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NATIONAL CANCER INSTITUTE

ANNUAL REPORT

October 1, 1977 through September 30, 1978

Part III-b

DIVISION OF CANCER CAUSE AND PREVENTION

ANNUAL REPORT

CARCINOGENESIS RESEARCH PROGRAM
DIVISION OF CANCER CAUSE AND PREVENTION
US NATIONAL CANCER INSTITUTE

Report of Program Activities

October 1, 1977 through September 30, 1978

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1. OVERVIEW

This report is probably the last from the Carcinogenesis Research Program, a short-lived program formed in 1976 when the Carcinogenesis Program was divided into the Carcinogenesis Testing Program and the Carcinogenesis Research Program. The Program has not had at any time a fully committed permanent Director. On an emergency basis in the autumn of 1977 a committee of the various Branch Chiefs within the Program was formed to act in lieu of a Director. In addition one of the members of the group was assigned signatory authority and other responsibilities as an Acting Associate Director. According to the reorganization of the Division under a new Director, the Carcinogenesis Research Program as an entity will disappear. Despite the lack of a permanent leader with the resultant uncertainty, the Carcinogenesis Research Program has continued to function as evidenced by the 123 publications from the intramural area alone, and 73 resulting from collaboration between intramural and contract groups.

In addition the members of the intramural group constitute a body of expertise in chemical carcinogenesis, a valuable resource at present when chemical carcinogenesis is so much in the public view. Personnel from the intramural program have served as consultants for Government agencies and numerous other groups on a national and international basis. Besides serving as a resource, the intramural staff has been at the forefront of research which represents advances in such areas as prevention of cancer and delineating the molecular and biological mechanisms of carcinogenesis.

2. SCIENTIFIC OVERVIEW AND PROGRESS HIGHLIGHTS

The objectives of the Division, aptly Division of Cancer Cause and Prevention, are to determine the causes of cancer in humans and to determine means to prevent cancer. Correspondingly, the efforts of the Carcinogenesis Research Program can be divided into these two areas, namely to determine the causes of cancer and the mechanisms by which it occurs, and to study means to prevent the transformation from a normal to a malignant state.

A. CAUSE

Biological models - short term and in vitro tests:
The use of various types of cells in culture as a potential bioassay system is being investigated in several sections of the Program. Human fibroblasts in culture, when treated with a promoting agent such as a phorbol ester, underwent transformation earlier but did not yield a stable malignant transformation. Although xeroderma pigmentosum cells were not very sensitive to transformation to 4-nitroquinoline-1-oxide, they were sensitive to the cytotoxic effect of various carcinogens (Chemistry Branch). Normal and xeroderma pigmentosum human fibroblasts were also used to study the role of DNA repair in mutagenesis and carcinogenesis caused by ultraviolet radiation (Biology Branch).

The Biology Branch continues to study host mediated fetal hamster cells as a system for identifying carcinogens. Certain carcinogens showed a zero threshold

level, others a dose response relationship in this system. The environmental carcinogen vinyl chloride transformed cells in this host mediated assay but was not effective when applied directly to cells. This indicates that an activated metabolic product was probably responsible (Biology Branch).

It was concluded that the process of carcinogenesis in hamster cells occurs in two discrete steps. New methods for the transfer of markers involved in carcinogenesis were developed using either chromosomes or DNA (University of Ontario, CP-43331).

Fetal guinea pig cells in culture were used to study stages from the growth controlled-to the neoplastic state. Carcinogenesis occurred independently of the age and number of cell divisions of the target cells. Neoplastic transformation of diploid human cells was obtained by treating the cultured cells in the "S" phase of the cell cycle with several types of carcinogens (Biology Branch).

Other cell systems in use include rat liver epithelial cells where growth in agar and cytologic markers were considered valid indicators of transformation. Tumor promoting phorbol esters induced plasminogen activator in cell cultures and produced changes in cell phenotype which mimic those of transformed cells (Columbia University, CP-23234). Similarly, phorbol esters stimulated the uptake of choline and ethanolamine into the corresponding phospholipids of rat liver cells into culture. The LDH isoenzymes from hepatocytes transformed by nitrosomethylurea were less stable than those from normal hepatocytes (Carcinogen Metabolism and Toxicology Branch). Mouse epidermal cell cultures of varying degrees of differentiation were obtained after treatment with different types of carcinogens (Experimental Pathology Branch).

Mutagenesis and its significance in carcinogenesis are being followed by several contract groups. The University of Ontario (CP-43331) is using CHO cells as a system to obtain recessive mutants in order to investigate the genetic mechanism. New York University Medical School (CP-33279) has a mutant hamster cell line which can be arrested in a specific phase of the cell cycle analogous to the situation in malignant cells.

Cell mutants with altered levels of the enzymes involved in pyrimidine nucleotides have been used to study normal regulation of pyrimidine nucleotide synthesis (University of California, San Francisco, CP-43239). Johns Hopkins University (CP-55713) has induced somatic mutations and morphological transformation in cells by treatment with bromodeoxyuridine and ultraviolet radiation. The only cellular target seemed to be the DNA.

Benzopyrene, 3-methylcholanthrene and dimethylbeenzanthracene were highly effective mutagens of equal potency when compared at similar extents of in vivo DNA reaction. Therefore, their varying carcinogenic potency is probably related to their different degrees of metabolism and DNA reaction (Institute of Cancer Research, CP-33367).

DNA repair and reaction has been used as a means of indicating the possible carcinogenic potency of compounds. Using such a system it was found that a human colon cancer cell in culture had a reduction of 50-70% of the normal

repair, compared to normal human fibroblasts (Oak Ridge, CP-50200). Anti-benzopyrene diol epoxide I, an ultimate carcinogen, leads to gaps in newly replicating DNA which are ultimately closed by a caffeine-sensitive repair mechanism. The less carcinogenic syn-benzopyrene diol epoxide II produces 2 gaps per bound molecule which were eventually closed (Experimental Pathology Branch). At the Frederick Cancer Research Center (FCRC) (CO-75380) it was noted that major differences in the repair response to chemicals of different carcinogenic potency were not detected.

Another short term test, but an in vivo one, is being conducted by the University of California, San Diego (CP-33232). Certain organohalides carrying a nucleophilic substituent showed a significant tumorigenic activity in strain A mice compared with others which did not carry such groups. Caffeine had a suppressive effect on spontaneous and urethane induced lung tumors in strain A mice. This may be due to a general suppression of DNA synthetic activity.

Tumor Models

Under the Program several effective animal models simulating human tumors such as those of the colon have been developed. In this program the American Health Foundation (CP-33208) has found that a high fat diet fed to rats also given a colon carcinogen increases the yield of colon cancer, perhaps through a higher level of bile acids. Dietary fibers such as wheat, bran or pectin counteracted the enhancing effect of dietary fat. An epidemiolcgical study on human populations in rural Finland compared to those from higher risk New York seems to substantiate the conclusion from the animal study.

Lithocholic acid, a major human bile acid, enhanced the mutagenicity of known carcinogens. Likewise, metabolism of environmental dyes by human intestinal flora produced mutagenic substances (FCRC-CO-75380).

Various techniques for development of lung cancer in animals have been followed. There was a synergistic effect in animals following sequential exposure to low doses of 210 Po and benzopyrene. Saline instillation stimulated the cell proliferation in the lung after the initial exposure to carcinogen. Thus this inoccuous stimulation potentiated lung carcinogenesis; an observation which may have important implications in terms of the exposure to cigarette smoke and alpha radiation in human populations (Harvard School of Public Health, CP-33273).

Bis-(chloromethyl)ether, a known carcinogen, may be formed from hydrogen chloride and formaldehyde. Rats exposed to 10 ppm of each of these compounds developed carcinomas of the nasal cavity. This could have significant implications for industrially exposed populations. Numerous industrial materials in dusts have carcinogenic or cocarcinogenic activity in the respiratory tract (New York University, CP-33260).

In organ cell cultures multistage carcinogenesis was demonstrated in tracheal epithelium. In addition nickel subsulfide was a weak but definite carcinogen for respiratory epithelium (Oak Ridge, CO-50200).

Metabolic alterations developed in the epithelium of tracheal explants after addition of polycyclic aromatic hydrocarbons to the culture medium. The

possible enhancing effects of inorganic particles such as ferric oxide and crocidite asbestos along with methylcholanthrene are being investigated. Exposure of tracheal organ cultures to asbestos caused both hyperplastic and metaplastic epithelial changes (University of Vermont, CP-33360).

An excellent model for pancreas cancer has been developed in Syrian hamsters using N-nitroso-bis(2-oxypropyl)- and -(2-hydroxyl-propyl)amine. These compounds seemed to be especially effective for the pancreas and few other tumors were induced. However, although N-nitroso-bis-(2-oxypropyl)amine is a potent pancreas carcinogen in hamsters, it induced colo-rectal cancer in a high proportion of MRC rats receiving a weekly injection. The tumor morphology closely resembled the corresponding human disease (Eppley Institute, CP-33278).

A canine model was found appropriate to study the pathogenesis and role of reflus of duodenal contents into the pancreatic duct. The gastrointestinal hormones may be an important factor in modulating bile flow and in induction of reflux (Mayo Foundation, CP-55660; and University of Utah, CP-55709).

The propagation normal pancreatic exocrine cells was accomplished by cocultivation of the exocrine cells with irradiated fibroblasts (American Type Culture Collection, CP-65751). On the other hand a method for isolating pancreatic ductal cells from whole bovine pancreas was developed at the University of Maryland (CP-75947).

In the area of studies on prostatic cancer, a profile of histochemical markers specific for rat ventral prostatic epithelium, has been developed. These markers have been applied in primary cultures to identify prostatic epithelial cells (W. Alton Jones Cell Science Center, CP-65762). An animal model has been discovered; aged male A X C rats develop prostatic tumors spontaneously. The morphogenesis of the lesion is being investigated.

Although not a specific tumor model, injection of ethylnitrosourea into pregnant female Erythrocebus patas led to various tumors in the offspring which closely resembled the pediatric tumors in man. Furthermore some adult females given ethylnitrosourea during pregnancy have died within 6 months from metastatic choriocarcinoma. This is the first experimental model for systemic induction of choriocarcinoma, a neoplasm which is not inducible in rodents by carcinogens (Experimental Pathology Branch).

Human Tissue Studies

Model systems have been developed for human bronchus, colon, esophagus, lung and pancreatic duct. These models have the advantage that the effects of carcinogens can be observed directly in the human target tissues without need for extrapolation from a rodent model. Various polynuclear aromatic hydrocarbons, nitrosamines, hydrazines, and aflatoxin B_1 were all metabolically activated to forms that bound to macromolecules such as DNA. In some cases the major reactive metabolites and their adducts could be identified. There was a 75-fold variation in binding levels of benzopyrene to DNA in bronchial specimens from 100 patients. Although there was a 5-fold difference in the binding of benzopyrene to DNA among the different anatomical segments from the colon of the same patient, the binding level was generally highest in the

ascending colon (Experimental Pathology Branch).

Viable human esophageal epithelium could be maintained in explant cultures for a period of 6 months (University of Maryland, CP-75909). The FCRC (CO-75380) has developed a method to isolate and grow large numbers of normal breast epithelial cells from human tissue. The availability of such cultures will allow the investigation of the effects of hormonal levels and other factors on morphological alterations in the cells.

Application of dimethylbenzanthracene or N-methyl-N'-nitro-N-nitrosoguanidine to normal breast epithelium in culture led to changes which closely resembled dysplastic epithelium or lobular carcinoma, as seen in vivo. Similarly, application of a mixture of benzopyrene and ferric oxide to cultures of normal human bronchial epithelium led to changes resembling carcinoma in situ (University of Maryland, CP-43237).

Molecular Mechanisms and Metabolism.

Certain non-histone DNA binding proteins from the nucleus of malignant cells differed from cell proteins in normal cells. An immunological approach has been developed to check the localization of these proteins (Vanderbilt University, CP-65730). Transfer RNA isolated from animals treated with ethionine were remarkably impaired in their capacity to sustain protein synthesis, as detected by injection into Xenopus oocytes. However, the changes were so subtle that they could not be detected by convential physicochemical means (Weizmann Institute, CP-33220).

Irradiated thyroid cells injected intraperitoneally into isogenic recipient fish resulted in thyroid tumors, probably due to migration of the injected cells to the thyroid and concentration there (Brookhaven Labs., CP-50202).

The role of surface membranes in the process of carcinogenesis is under investigation. The mitochondria from a number of transformed tumor cell lines had a high affinity for calcium and retained it very tenaciously. The observation may help explain why certain human cancers preferentially spread to the bones (Johns Hopkins, CP-45610). The elevation in phospholipids found in transformed hamster cells in cultures is due to a new component, phosphatidyl threonine. This effect was not seen in the mouse and the chick indicating that it is a species specific response of the hamster cell (University of Rochester, CP-45611).

Metabolism.

At the University of Wisconsin (CP-65734) it was found that there was an excellent correlation between metabolic rate for benzopyrene in nitrogen-stimulated lymphocytes and the half-life of antipyrine in vivo. The results tend to confirm that persons with genetically controlled increases in AHH activity are at greater risk of lung cancer. Patients with bronchogenic carcinoma or oral-pharyngeal carcinoma had higher AHH inducibility to the normal donors (University of Texas, CP-55604). Under this contract the lymphocyte culture AHH assay has been improved to a point where it has become an accurate and reproducible research tool. In contrast, at Roswell Park Memorial Institute

(CP-55629) no difference in AHH activity of inducibility between lung cancer patients and matched controls was found.

Various cytochrome P-450's which exhibited different catalytic activities in the metabolism of benzopyrene and the (-) trans 7,8-diol of benzopyrene were separated. Methods for analysis of the various benzypyrene metabolites have been developed employing high pressure liquid chromatography (Chemistry Branch).

The Institute of Cancer Research (CP-33367) has evidenced from the metabolism of 3-methylcholanthrene which also provides support for the bay region diol-epoxide concept of hydrocarbon activation. A project at the University of Chicago (CP-33385) has accomplished the synthesis of diol-epoxide derivatives of a series of carcinogenic polycyclic hydrocarbons. The bay region diol epoxide derivatives of certain of these compounds exhibit significant biological activity. Methods were also developed to resolve the dihydrodiol metabolites of benzopyrene by means of high pressure liquid chromatographic separation of certain derivatives. These studies were necessary to assign the absolute configuration of the metabolites bound to DNA in vivo.

A comparative metabolic study of benzopyrene in microsomal fractions from hamster, human and rat liver and lung showed that human lung formed a two-fold larger fraction of the 7,8- and 9,10-dihydrodiols than did those from hamster and rat tissue (University of Texas, CP-33362).

The FCRC (CO-75380) has developed high temperature nematic liquid crystals for gas-liquid chromatographic separations of mixtures of polynuclear aromatic hydrocarbons and their metabolites. Studies with dimethylbenzanthracene indicated that a diol epoxide was generated in the 1,2,3,4 ring and not in the 8,9,10,11 ring. The findings support the generalization of Jerina and Daly which suggest that hydrocarbons are activated through the bay region diol epoxide. In microsomal systems it was observed that a reactive K region epoxide of dimethylbenzanthracene was produced and not a 1,2,3,4 ring diol-epoxide. These results suggest that the oxidative metabolism of polycyclic aromatic hydrocarbons by liver microsomes may differ from the pathway in target tissues. Thus the use of liver microsomes as an activating system in mutagenicity assays may be misleading.

Midwest Research Institute (CP-32270) has found that the major metabolic site for 3-methylcholanthrene is the 1-position as indicated from in vivo and in vitro studies in liver and pancreas. Metabolism at the 11,12-position was secondary.

The important industrial intermediate,e, o-toluidine, led to many urinary metabolites. However, the only one of these metabolites which was mutagenic was 2-nitrosotoluene (American Health Foundation, CP-55639).

At Southern Research Institute (CP-55721), the metabolism of various halogenated compounds of environmental use such as 1,2-dibromoethane and 1,2-dibromo-3-chloropropane was investigated. Both these compounds were substrates for glutathione transferase. 2-Bromoethanol was a metabolite of 1,2-dibromoethane.

The metabolism of several aromatic amines of environmental importance is being followed in the Carcinogen Metabolism and Toxicology Branch. The hair dye intermediate 2,4-diaminoanisole was largely detoxified through acetylation on both amino groups. One urinary metabolite was identified where oxidation of the acetyl side chain to an alcohol had occurred.

Identification of Environmental Carcinogens.

A contract at the University of Hawaii (CP-75915) has as its aim the development of analytical procedures for cycasin and macrozamin in meat. Since meat from steers which may have eaten forage containing these toxins is shipped frozen to this country for human consumption, the need for such a method is evident. Despite many interfering substances in meat, a gas chromatographic method was developed to determine such compounds when added directly to meat. It will now be extended to steers which have been fed cycads directly before slaughter.

At the University of Missouri (CP-75946) it was found that a retroaldol type fragmentation of bis(2-hydroxyethyl)nitrosamine may occur under conditions simulating those found in cutting fluids at the metal-tool interface in a metal grinding operation. Unfortunately the products are dimethylnitrosamine, N-nitrosomorpholine, methylvinylnitrosamine, 2-hydroxyethylmethylnitrosamine and 2-hydroxyethylvinylnitrosamine compounds which are more carcinogenic and thus more hazardous than the starting hydroxyethylnitrosamine. It was also noted that a recommended method for analysis of nitrosamines in foodstuffs led to production of nitrosamines which are artifacts and may lead to misleading information for regulatory agencies or epidemiological studies.

In gastric juice from individuals living in a high risk area for gastric cancer in Colombia, many cases with very high levels of nitrite were found, leading to the possibility of formation of nitrosamines. However, ingestion of nitrite in foodstuffs is not the only source for its formation. Exogenous formation of nitrite and nitrate can occur in the small intestine of man, probably by heterotrophic nitrification of ammonia. These results indicate that nitrosamine formation may be an important factor than previously realized for colon carcinogenesis in humans (Massachusetts Institute of Technology, CP-33315).

B. PREVENTION

In this area there have been some relatively new developments which make more hopeful the possibility that cancer in humans can actually be prevented. The Lung Cancer Branch is active in this area, employing retinoids or derivatives of vitamin A. In hamster tracheal organ cultures vitamin A analogs could be used to reverse the keritinization process in retinoid deficient trachea or in trachea which were in the process of transforming to a metaplastic state. Various synthetic analogs of vitamin A were much less toxic than the natural form of the vitamin. One of the metabolites of retinoic acid in culture has significant biological activity in reversing the keritinization process.

The use of retinoids in inhibiting or suppressing carcinogenesis in other epithelial type cells is under investigation. For example, at the Illinois Institute of Technology Research Institute the use of retinoids in suppressing DMBA-induced mammary cancers in rats is being studied (CP-23292).

Microbiological Associates (CP-02199) in collaboration with NCI staff, is following the use of retinoids in inhibiting nitrosomethylurea-induced bladder cancer.

In order to continue these valuable studies, several new contracts have been initiated on the synthesis of analogs of retinoids and to supply quantities of retinoids sufficient for both animal and possible clinical testing. A workshop on chemoprevention of cancer was held in February at which some of the problems related to inhibition of formation or activation of carcinogens were explored. However, other means are also being investigated to prevent the effects of chemical carcinogens. For example, at the University of Minnesota (CP-33364) it was shown that the antioxidant butylated hydroxytoluene alters the microsomal metabolism of carcinogens and enhances their detoxication so that pulmonary neoplasia is decreased. The microsomal-catalyzed covalent binding of the 7,8-dihydrodiol of benzopyrene to DNA was inhibited in the presence of glutathione and glutathione transferase. Although both BHT and L-ascorbic acid were protective agents, L-ascorbic acid had no effect on DNA binding.

Tryptophan can inhibit to some extent carcinogens such as dimethylnitrosamine and 4-dimethylaminoazobenzene. It was found that tryptophan metabolites which underwent autoxidation to phenoxazine type compounds could inhibit azoreductase, an important enzyme, in detoxication of azo dyes (Carcinogen Metabolism and Toxicology Branch).

The prevention of chemical carcinogenesis by dietary means is also possible. A contract at Massachusetts Institute of Technology (CP-33238) has shown that lipotrope or amino acid deficiencies can enhance induction of hepatocarcinoma by diethylnitrosamine and that high fat can enhance induction of dimethylhydrazine colon tumors. Further studies on these dietary factors will permit definition of components which retard chemical carcinogenesis.

C. RESOURCES FOR CARCINOGENESIS RESEARCH.

In order to aid carcinogenesis research, a contract with the Illinois Institute of Technology Research Institute (CP-55646) provides reference samples of chemical carcinogens of known purity. In view of the recent increased interest in synthetic fuels from coal and similar sources, new compounds which have been found to occur in such fuel sources are being added. Analytical data as well as information on the safe handling of each chemical is provided with each shipment of compounds from the repository. The program also provides other resources such as animals and information services to qualified investigators.

Carcinogenesis Publications

Total Number of Publications in FY 1978

Program	Total No.	Per Cent
Authorship	759	100.0%
(a) INTRAMURAL PROGRAM Exclusively intramural staff	123	16.0%
(b) COLLABORATIVE PROGRAM Exclusively contract-supported investigators	564	74.0%
(c) INTRAMURAL/COLLABORATIVE PROGRAM Joint	72	10.0%

3. ORGANIZATIONAL STRUCTURE AND MANAGEMENT

The organization of the Carcinogenesis Research Program is presently divided into two major components: intramural program and collaborative program. The former is articulated in the Office of the Associate Director and in five branches, each with several sections and units. The latter is articulated into three operational units comprised of a total of seven segments. Each operational unit is directed by a scientific program manager. Complementing segments, which together comprise an operational unit, are each directed by a senior staff member; other members of the intramural staff serve as project officers on individual contracts. Segments are assisted by individual ad hoc advisors from outside the NCI. One technical peer review committee provides expert review of specific project plans and proposals submitted to the program. It provides recommendations concerning the scientific merit of the research and technical competence of the proposed staff prior to the award of contracts or other appropriate support instruments.

This section is divided into the following four units:

A. Management and Review System

B. Diagram of the Collaborative Research Organization

C. Diagram of the Intramural Organization

D. Listing of Advisory Groups and Consultants within the Carcinogenesis Research Program

A. MANAGEMENT AND REVIEW SYSTEM

The Carcinogenesis Research Program is responsible for planning, directing, coordinating and evaluating a program of basic and applied research on the cancer-causative factors and the prevention of carcinogenesis; for establishing program priorities, allocating resources, integrating the projects of the various branches, evaluating program effectiveness, and representing the program area in management and scientific decision-making meetings within the Institute; for administering research, through intramural laboratories and contracts, in carcinogenesis and related toxicology, metabolism, chemistry, immunology, cell biology, experimental tumor pathology, and information sciences; and for advising the Director of the Division of Cancer Cause and Prevention and for supporting the activities of the National Cancer Advisory Board and other scientific advisory committees.

The organization of the Carcinogenesis Research Program is divided into two major components: An intramural program and a contract-supported collaborative program. The intramural program is primarily devoted to laboratory research, scientific documentation, and program coordination and consists of the Office of the Associate Director for Carcinogenesis Research (including the Office of the Coordinator for Collaborative Research) and five branches (Biology, Carcinogen Metabolism and Toxicology, Chemistry, Experimental Pathology, and Lung Cancer) each with several sections and units.

The collaborative program is devoted primarily to targeted research implemented through the contract mechanism and consists of three operational units comprised of six segments (Biological Models, Carcinogen Metabolism and Toxicology, Chemistry and Molecular Carcinogenesis, Colon Cancer, Information and Resources, and Lung Cancer). There is no programmatic distinction between the intramural and collaborative program except from an administrative standpoint. They are, in fact, designed to complement each other.

The Office of the Coordinator for Collaborative Research is comprised of a management team of scientist-administrators with both research and administrative experience. These scientist-administrators, designated as program managers (1) develop and implement improved management methods in the contract-supported collaborative programs; (2) assist in allocations of resources and evaluation of priorities in the overall program; and (3) coordinate the planning, administration, and evaluation of contractual research. In addition, each of the segments has a director, who provides scientific leadership, participating senior intramural staff who serve as project officers and provide technical guidance and direction. Peer review of proposals is provided by the Carcinogenesis Program Scientific Review Committee, whose members and chairman are from the outside scientific community. Review for priority, need, and relevance is performed by the Carcinogenesis Contract Program Management Group (CCPMG), a senior staff committee composed of representatives from all the major elements of the Program.

The following operating procedures are used to process New, Renewal, and Unsolicited Proposals:

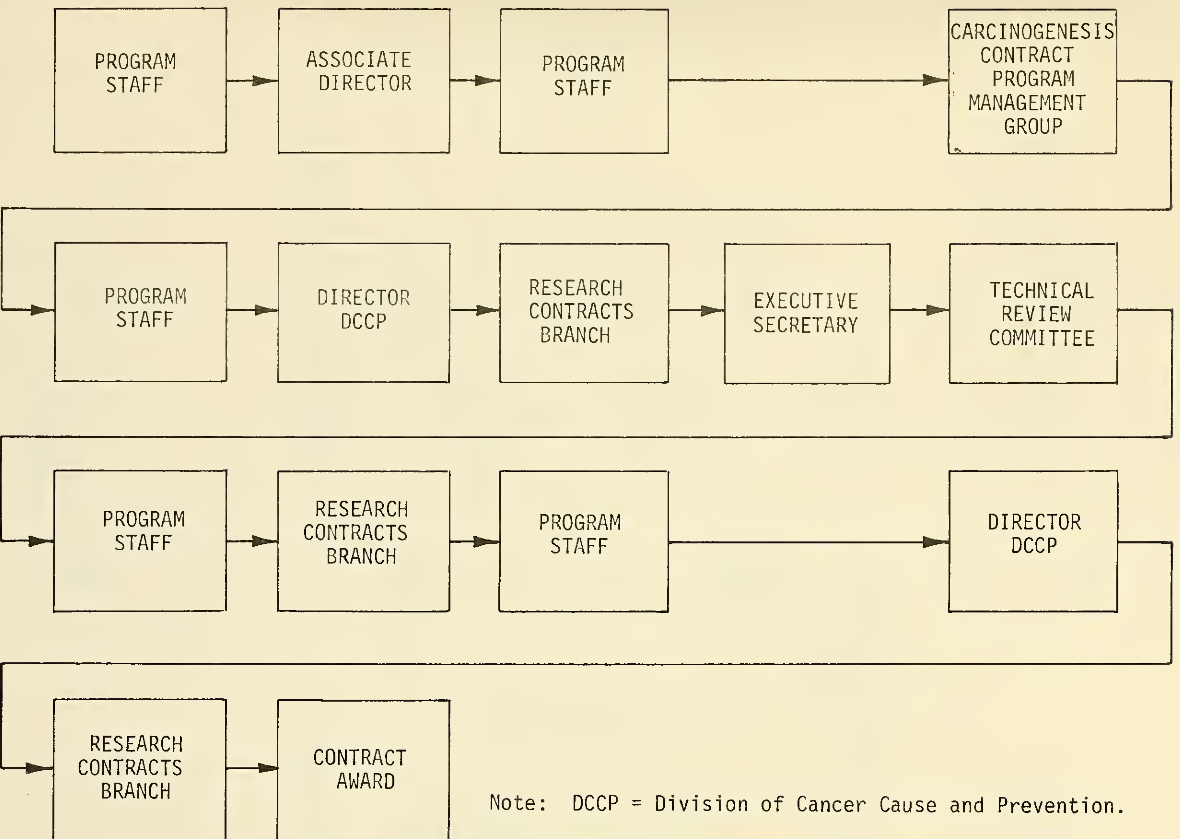
New Projects (Competitive Awards) (Figure 1) - New project ideas come primarily from the CCPMG, program staff, consultants, and workshops. A project plan will be prepared and submitted to the CCPMG for a review of Program relevance. If the project plan is approved, a request for proposal (RFP) will be prepared by the program staff. The research contracts branch (RCB) will advertise the RFP, and accept proposals. These will be evaluated by the technical review committee and ad hoc consultants. Based on the committee's recommendation, a contract will be negotiated and awarded.

Project Renewals (Figure 2) - The procedures for renewing projects are basically the same as initiating a new proposal, except that no new project plan is required. A technical evaluation is made of the renewal proposal; based on the review committee recommendations, a renewal contract will be negotiated and awarded.

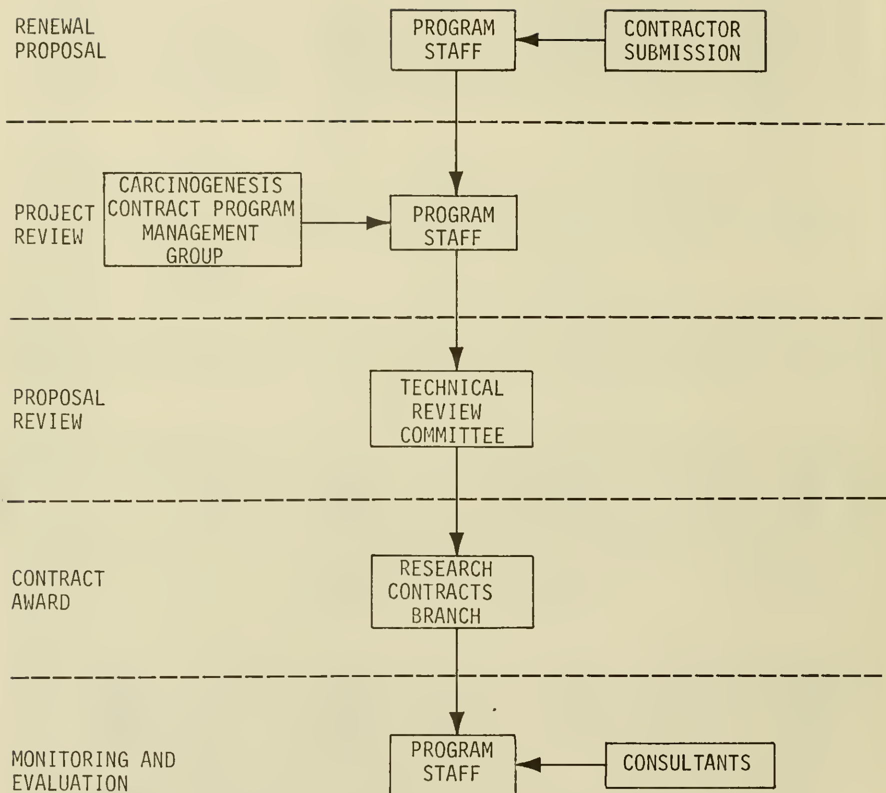
Unsolicited Proposals (Figure 3) - Unsolicited proposals are reviewed by the program staff for relevance, and if approved, will then be reviewed by the technical review committee. Based on the review recommendations, a decision will be made to negotiate and award a research contract.

As always, major requirements of time and effort have gone into the daily operational management involved in planning and obtaining approval of new programs, development of project plans, development and issuance of requests for proposals, proposal review, contract monitoring, and meeting ever-increasing requirements for documentation under the Freedom of Information Act and the Privacy Act. The major management challenge which remains is to develop and maintain a scientifically sound program in a broad multi-disciplinary field with very limited staff resources.

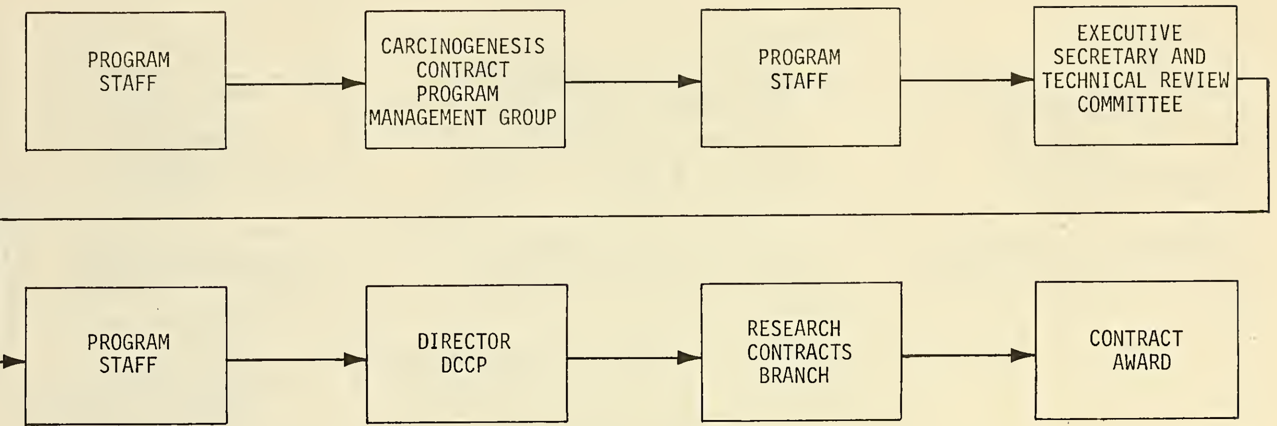
The staff has met this challenge but it is increasing its serious over-extension to a point which raises the question of how long the present pace can be maintained. Recognition is due the Carcinogenesis Research Program staff to whose extraordinary, invaluable, and untiring efforts this program owes its development. In spite of the continued imposition of strict limitations in personnel positions, the Program has maintained an excellent, highly productive intramural research activity together with a rapidly expanding collaborative program; both were made possible by the highly dedicated, often strenuous commitment of the members of the staff.



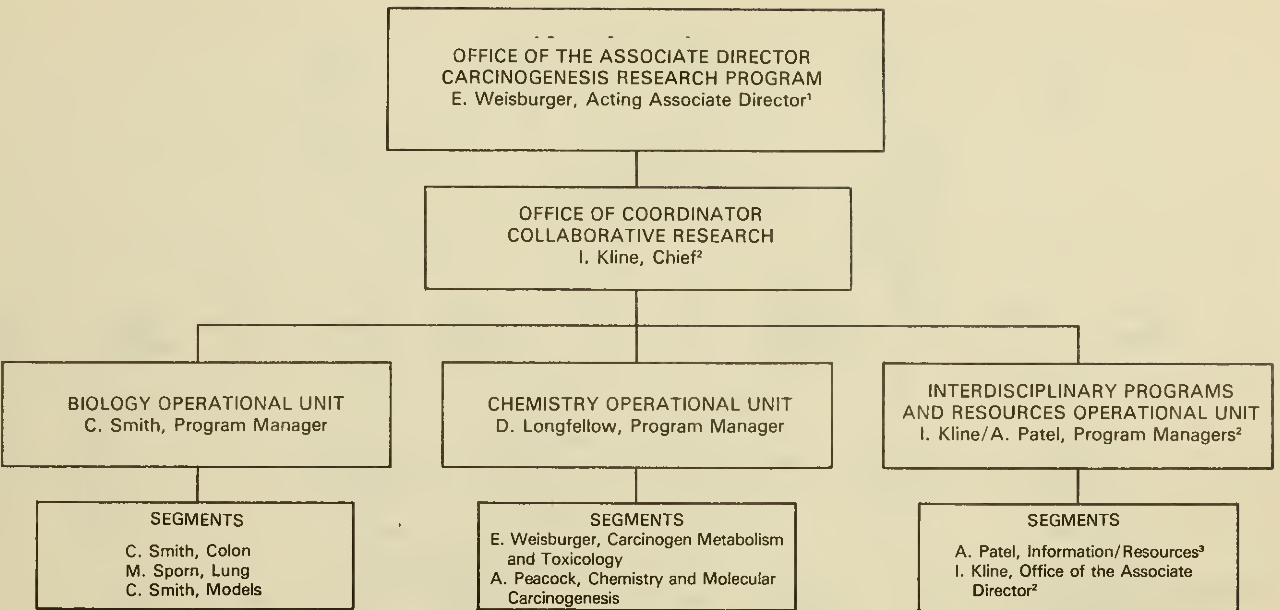
NEW PROJECTS (COMPETITIVE AWARDS)
(Figure 1)



PROJECT RENEWALS
(FIGURE 2)

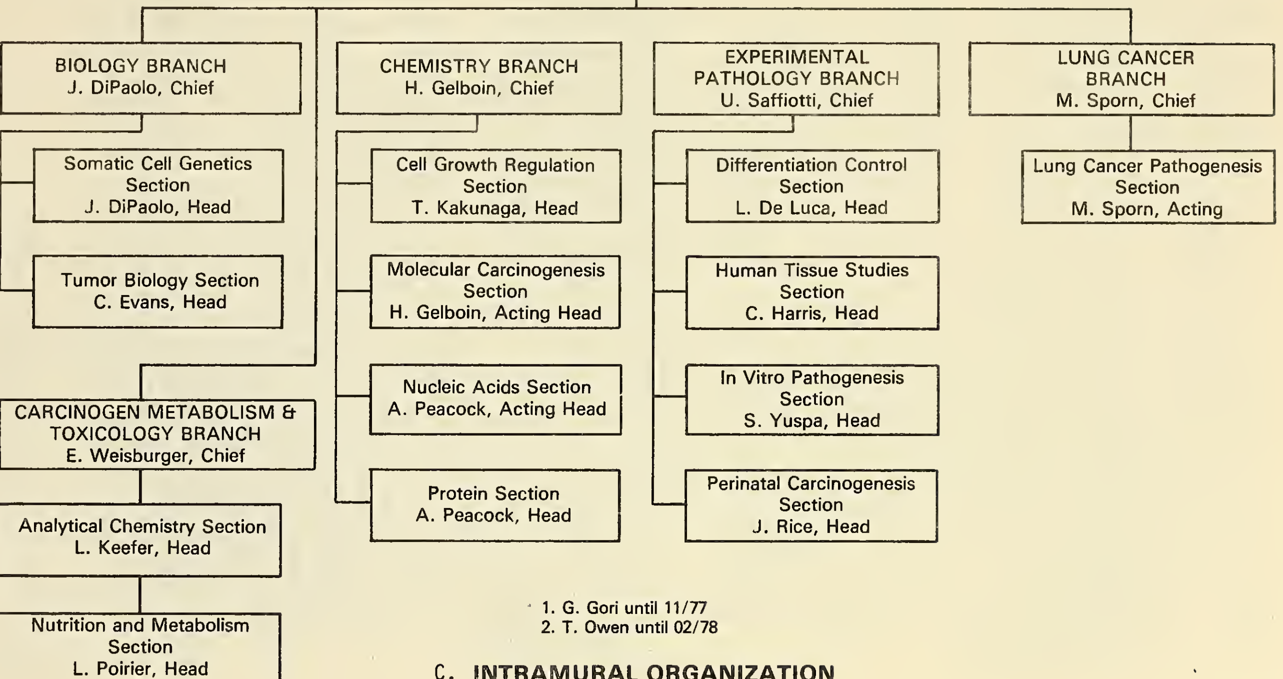


UNSOLICITED PROPOSALS
(Figure 3)



1. G. Gori until 11/77
2. T. Owen until 02/78
3. T. Owen until 06/77

B. **COLLABORATIVE RESEARCH ORGANIZATION**



1. G. Gori until 11/77
2. T. Owen until 02/78

C. **INTRAMURAL ORGANIZATION**

D. ADVISORY GROUPS AND CONSULTANTS

(1) The Branch Chiefs Group Committee was established in October of 1977. It provides a coordinating and planning function to the intramural program at the senior staff level. Since the vast majority of the intramural professional staff is now active in the planning and monitoring of the overall program, this group provides a vital interface between intramural and collaborative research programs. Membership of this group is limited to the staff of the Office of the Associate Director, the Administrative Officer, and the five branch chiefs.

Dr. Elizabeth K. Weisburger, Acting Associate Director, and
Chief, Carcinogen Metabolism and Toxicology Branch, *Chairman*
Ms. M. Joellyn Mesa, *Executive Secretary*
Dr. Joseph A. DiPaolo, Chief, Biology Branch
Dr. Harry V. Gelboin, Chief, Chemistry Branch
[1]Dr. Ira Kline, Chief, Office of the Coordinator for
Collaborative Research
Dr. Umberto Saffiotti, Chief, Experimental Pathology Branch
Dr. Michael B. Sporn, Chief, Lung Cancer Branch
Ms. Joan C. Topalian, Administrative Officer

(2) The Carcinogenesis Contract Program Management Group is a committee of staff scientists who provide review of proposed activities for priority, need, and relevance within the Carcinogenesis Research Program. Membership of this group consists of representatives of all the major elements in the Program: the Office of the Associate Director, the branch chiefs from the intramural scientific staff, segment directors from collaborative program scientific staff, as well as a representative from the Division of Cancer Research Resources and Centers.

[2]Dr. Elizabeth K. Weisburger, Acting Associate Director, and
Chief, Carcinogen Metabolism and Toxicology Branch, *Chairman*
Ms. A. Christine Manuel, *Executive Secretary*
Dr. Joseph A. DiPaolo, Chief, Biology Branch
Dr. Thaddeus Domanski, Program Director for Carcinogenesis,
Division of Cancer Research Resources and Centers, NCI
Dr. Harry V. Gelboin, Chief, Chemistry Branch
[1]Dr. Ira Kline, Chief, Office of the Coordinator for
Collaborative Research
Dr. Andrew C. Peacock, Director, Chemistry and Molecular
Carcinogenesis Segment
Dr. Umberto Saffiotti, Chief, Experimental Pathology Branch
Dr. Michael B. Sporn, Chief, Lung Cancer Branch

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[1]Dr. Thomas B. Owen until February, 1978
[2]Dr. Gio B. Gori until November, 1977

(3) The Carcinogenesis Program Scientific Review Committee provides expert peer review of proposals submitted to the Carcinogenesis Research and Carcinogenesis Testing Programs of the Division of Cancer Cause and Prevention, National Cancer Institute. Dual Executive Secretaries are appointed to represent both Program Areas. Based on an evaluation of the scientific merit of the proposed work and on an evaluation of the technical competence of the proposed staff, the Committee provides recommendations concerning the award of contracts or other appropriate support instruments.

- Dr. Robert E. Greenfield, National Bladder Cancer Project, St. Vincent Hospital, Worcester, MA, *Chairman*
- Dr. Virginia C. Dunkel, *Executive Secretary (Testing Program)*
- Dr. Carl E. Smith, *Executive Secretary (Research Program)*
- Dr. Gerald L. Bartlett, College of Medicine, Pennsylvania State University, Hershey, PA
- Dr. Howard A. Bern, Cancer Research Laboratory, University of California, Berkeley, CA
- Dr. Louis M. Fink, University of Colorado Medical School, Denver, CO
- Dr. Phillip Issenberg, Eppley Institute for Research in Cancer, University of Nebraska, Omaha, NB
- Dr. M. Edward Kaighn, Pasadena Foundation for Medical Research, Pasadena, CA
- Dr. Louise S. Lombard, Argonne National Laboratory, Argonne, IL
- Dr. Prabhakar D. Lotlikar, Fels Research Institute, Temple University School of Medicine, Philadelphia, PA
- Dr. Danuta Malejka-Giganti, Veterans Administration Hospital, Minneapolis, MN
- Dr. Roy Ritts, Jr., Mayo Graduate School of Medicine, Rochester, MN
- Dr. Evelyn M. Rivera, Michigan State University, East Lansing, MI

Other consultants who have been utilized by the Program throughout this fiscal year as contract site visitors, reviewers, individual technical advisors, or lecturers are as follows:

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- Dr. Larry M. Allen, University of Miami School of Medicine, Miami, Florida
- Dr. Joseph Arcos, Tulane University School of Medicine, New Orleans, Louisana
- Dr. Colin Arlett, University of Sussex, Falmer Brighton, England
- Dr. R. J. Berry, Middlesex Hospital Medical School, London, England
- Dr. John Bertrum, Roswell Park Memorial Institute, Buffalo, New York
- Dr. W. Robert Bruce, Ontario Cancer Institute, Princess Margaret Hospital, Toronto, Ontario, Canada
- Dr. Homer Black, Baylor College of Medicine, Texas Medical Center, Houston, Texas
- Dr. Peter Borchert, University of Minnesota, Minneapolis, Minnesota
- Dr. Roswell K. Boutwell, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin
- Dr. Bill Brinkley, Baylor College of Medicine, Texas Medical Center, Houston, Texas

Dr. Bertram Cohen, Public Health Research Institute of the City of New York, New York, New York
Dr. Gerald Cohen, University of Surrey, Guildford, England
Dr. Allan Conney, Hoffmann-LaRoche, Inc., Nutley New Jersey
Dr. Thomas Dao, Roswell Park Memorial Institute, Buffalo, New York
Dr. Jerome De Cossee, Medical College of Wisconsin, Milwaukee, Wisconsin
Dr. Eugene De Sombre, University of Chicago, Chicago, Illinois
Dr. James Fanning, Clemson University, Clemson, South Carolina
Dr. Emerich Fiala, American Health Foundation Valhalla, New York
Dr. Margaret Fox, Christie Hospital and Holt Radium Institute, Manchester, England
Dr. DeWitt S. Goodman, Columbia University, New York, New York
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Dr. Albert Jonas, Yale University School of Medicine, New Haven, Connecticut
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Dr. Ronald Malt, Massachusetts General Hospital, Boston, Massachusetts
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Dr. Robert G. Meeks, Ohio State University College of Medicine Columbus, Ohio
Dr. Sidney Mirvish, Eppley Institute for Research in Cancer, University of Nebraska, Omaha, Nebraska
Dr. Richard Moon, IIT Research Institute, Chicago, Illinois
Dr. Gerald C. Muller, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin
Dr. Robert Palmer, The Rockefeller University, New York, New York
Dr. Malcolm C. Paterson, Atomic Energy of Canada Limited, Chalk River, Ontario, Canada
Dr.Anthony Pegg, Milton S. Hershey Medical Center, Hershey, Pennsylvania
Dr. Lynne Reid, Children's Hospital, Boston, Massachusetts
Dr. Seymour L. Romney, Albert Einstein College of Medicine, Bronx, New York
Dr. Jeffrey Rosen, Baylor University, Houston, Texas
Dr. James Selkirk, Oak Ridge National Laboratory, Oak Ridge, Tennessee
Dr. Sidney Silverman, Hood College, Frederick, Maryland
Dr. Vincent F. Simmon, Menlo Park, California
Dr. Jo W. I. M. Simons, State University of Leiden, Wassenaarsewg, The Netherlands

Dr. Andrew Sivak, Arthur D. Little, Inc., Cambridge, Massachusetts

Dr. Thomas Slaga, Oak Ridge National Laboratory, Oak Ridge, Tennessee

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Dr. Joseph Sperling, Weizmann Institute of Science, Rehovot, Israel

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Dr. John H. Weisburger, American Health Foundation, Valhalla, New York

Dr. Hiroshi Yamasaki, Columbia University, New York, New York

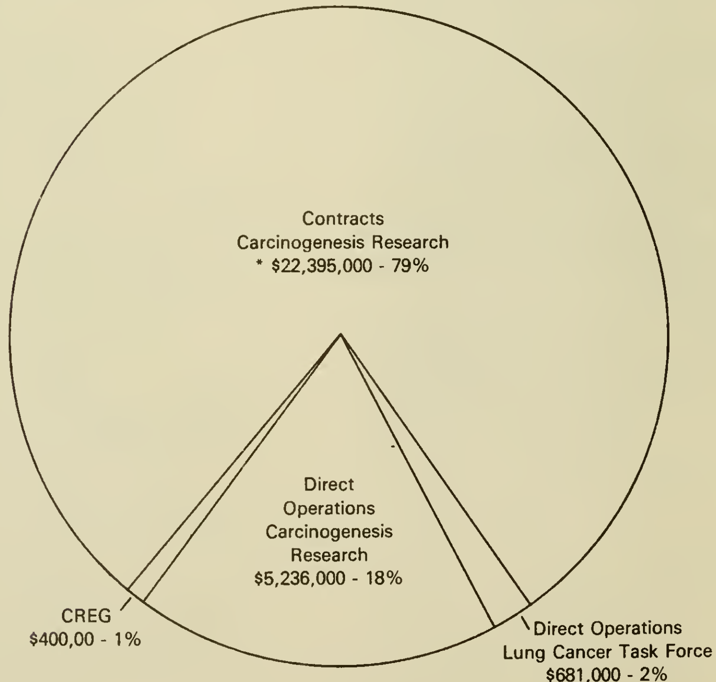
4. FISCAL INFORMATION

The following charts and tables report the present distribution of funds in the Carcinogenesis Research Program for Fiscal Year 1978, including Direct Operations and Contracts:

FIGURE 1 - Allocations - Direct Operations and Contracts
FIGURE 2 - Distribution of Contract Funds by Operational Units
TABLE I - Analysis of Contract Activities by Operational Units (with supporting Program Areas)
TABLE II - Analysis of Contracts by Operational Units

CARCINOGENESIS RESEARCH PROGRAM

Allocations - Direct Operations and Contracts



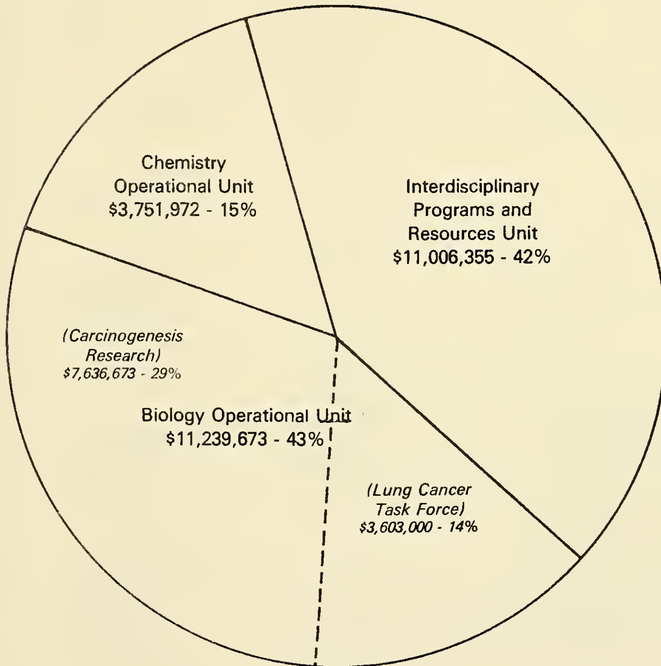
* does not include Lung Cancer Task Force funds for contracts in the amount of $3,603,000

FIGURE 1

CARCINOGENESIS RESEARCH PROGRAM

Distribution of Contract Funds by Operational Units

FISCAL YEAR 1978*



* Obligated and estimated funding information available as of April 10, 1978.

FIGURE 2

TABLE I

ANALYSIS OF CONTRACT ACTIVITIES BY OPERATIONAL UNIT (WITH SUPPORTING PROGRAM AREAS) IN THE CARCINOGENESIS RESEARCH PROGRAM

OPERATIONAL UNIT	NO. OF CONTRACTS	TOTAL AMOUNT*	PERCENT
TOTAL →	124	$25,998,000	(100)
BIOLOGY	74	11,239,673	43.3
Colon	11	1,534,207	5.9
*Lung	26	5,325,617	20.6
Models	37	4,379,849	16.8
CHEMISTRY	29	3,751,972	14.4
Chemistry and Molecular Carcinogenesis	19	2,531,228	9.7
Carcinogen Metabolism and Toxicology	10	1,220,744	4.7
INTERDISCIPLINARY PROGRAMS AND RESOURCES	21	11,006,355	42.3
Information/Resources	12	1,354,491	5.2
Office of the Associate Director	9	9,651,864	37.1

*Includes Lung Cancer Task Force funds in the amount of $3,603,000

TABLE II

ANALYSIS OF CONTRACTS BY OPERATIONAL UNITS
IN THE CARCINOGENESIS RESEARCH PROGRAM

UNIT CONTRACT	TITLE
1. BIOLOGY OPERATIONAL UNIT	
AICHI CANCER CENTER RESEARCH INSTITUTE (N01-CP-55650)	Induced Perinatal Alterations and Their Influence on Carcinogenesis
ALTON OCHSNER MEDICAL FOUNDATION (N01-CP-65764)	Study of the Influence of Repeated Low Dose Irradiation on Mammary Gland Carcinogenesis in Estrogenized Rats
AMERICAN HEALTH FOUNDATION (N01-CP-75952)	Studies of Colon Carcinogenesis in Organ Culture of Intestinal Mucosa
AMERICAN HEALTH FOUNDATION (N01-CP-75948)	Studies of Metabolic Capacity in Intestinal Mucosa
AMERICAN HEALTH FOUNDATION (N01-CP-75940)	Long-Term Studies of Prevention of Epithelial Cancer by Retinoids
AMERICAN HEALTH FOUNDATION (N01-CP-33208)	Studies in Colon Carcinogenesis
AMERICAN TYPE CULTURE COLLECTION (N01-CP-65751)	Development of Cell Strains from Ductal and Exocrine Portions of the Pancreas
BAYLOR COLLEGE OF MEDICINE (N01-CP-65837)	Markers for Evaluation of Preneoplastic Lesions in the Respiratory Tract
BIOTECH RESEARCH LABORATORIES, INC. (N01-CP-55640)	Criteria for In Vitro Cell Transformation by Viruses and Chemical Carcinogens
CALIFORNIA, UNIVERSITY OF (N01-CP-75934)	Synthesis of New Retinoids for In Vitro Studies of Lung Cancer and Other Epithelial Cancers
CHICAGO, UNIVERSITY OF (N01-CP-65777)	Markers for Evaluation of Preneoplastic Lesions in the Respiratory Tract
CHILDREN'S HOSPITAL OF LOS ANGELES (N01-CP-55641)	Fibrinolysis as a Parameter of In Vitro Transformation

TABLE II (CONTINUED)

UNIT CONTRACT	TITLE
COLORADO, UNIVERSITY OF (N01-CP-75875)	Tissue Interactions in Induction and Perpetuation of Hormonally-Induced Permanent Cellular Alterations
COLORADO, UNIVERSITY OF (N01-CP-65849)	In Vitro Cultivation of Normal Epithelial, Human Prostatic Cells
COLUMBIA UNIVERSITY (N01-CP-75897)	Synthesis of New Retinoids for In Vitro Studies of Lung Cancer and Other Epithelial Cancers
DARTMOUTH COLLEGE (N01-CP-65776)	Development of an Animal Model for Oat Cell Carcinoma of the Lung
DARTMOUTH COLLEGE (N01-CP-55708)	Chemical and Structural Requirements for Pancreas Uptake and Excretions: Rats and Hamsters
DARTMOUTH COLLEGE (N01-CP-33378)	Enhanced Delivery of Synthetic Nitroso Compounds to the Pancreas in Rats
DOE-NCI INTERAGENCY AGREEMENT (Brookhaven National Laboratory) (Y01-CP-60219)	Influence of Repeated Low Dose Irradiation on Mammary Gland Carcinogenesis in Estrogenized Rats
DOE-NCI INTERAGENCY AGREEMENT (Brookhaven National Laboratory) (Y01-CP-30213)	Synergistic Interaction of Hormones and Neutron Radiation of Mammary Gland Carcinogenesis
EXPERIMENTAL PATHOLOGY LABORATORIES, INC. (N01-CP-65763)	Resource for Microscopic and Autoradiographic Technology
GEORGIA, MEDICAL COLLEGE OF (N01-CP-55656)	Study of the Potential for Metabolic Activation in Animal Pancreas
GEORGIA, MEDICAL COLLEGE OF (N01-CP-43282)	Epidemiological Studies of the Incidence of Colon Cancer Among Blacks and Whites
HAWAII, UNIVERSITY OF (N01-CP-75933)	Synthesis of New Retinoids for In Vitro Studies of Lung Cancer and Other Epithelial Cancers
HARVARD UNIVERSITY SCHOOL OF PUBLIC HEALTH (N01-CP-33273)	Factors Influencing Experimental Respiratory Carcinogenesis by Alpha Radiation and Chemical Carcinogens

TABLE II (CONTINUED)

UNIT CONTRACT	TITLE
HOUSTON, UNIVERSITY OF (NO1-CP-75935)	Synthesis of New Retinoids for In Vitro Studies of Lung Cancer and Other Epithelial Cancers
IIT RESEARCH INSTITUTE (NO1-CP-75953)	Studies of Colon Carcinogenesis in Organ Culture of Intestinal Mucosa
IIT RESEARCH INSTITUTE (NO1-CP-75939)	Long-Term Studies of Prevention of Epithelial Cancer by Retinoids
IIT RESEARCH INSTITUTE (NO1-CP-43289)	Supply of Animals Treated with Epithelial Carcinogens
IIT RESEARCH INSTITUTE (NO1-CP-23292)	Studies of Modulating Factors in Epithelial Carcinogenesis
ILLINOIS, UNIVERSITY OF (NO1-CP-65843)	Maintenance and Scheduled Sacrifice of Guinea Pigs
INDIANA UNIVERSITY (NO1-CP-55654)	Chemical and Structural Requirements for Pancreas Uptake and Excretion: Rats and Guinea Pigs
KENTUCKY, UNIVERSITY OF (NO1-CP-75954)	Studies of Carcinogenesis in Human Tissues
LITTON BIONETICS, INC. (NO1-CP-65847)	Resource for Microscopic and Autoradiographic Technology
LITTON BIONETICS, INC. (NO1-CP-43274)	Studies of Carcinogenesis in Human Bronchial Tissues
MARYLAND, UNIVERSITY OF (NO1-CP-75947)	Isolation, Identification and Culture of Epithelial Cell Types from the Pancreas of Experimental Animals
MARYLAND, UNIVERSITY OF (NO1-CP-75909)	Studies of Carcinogenesis in Human Tissues
MARYLAND, UNIVERSITY OF (NO1-CP-43237)	Studies on Carcinogenesis in Human Tissues: Bronchial Epithelium, Pancreas, Breast, and Colon
MAYO FOUNDATION (NO1-CP-55660)	Reflux as a Mechanism for Induction of Adenocarcinoma of the Pancreas

TABLE II (CONTINUED)

UNIT CONTRACT	TITLE
MEDIZINISCHE HOCHSCHULE HANNOVER (NO1-CP-75972)	The Development of the European Hamster as an Animal Model for Pancreatic Carcinogenesis
MELOY LABORATORIES, INC. (NO1-CP-55613)	Transplacental Carcinogenesis in the Old World Monkey, Erythrocebus Patas
MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CP-43366)	Analysis of Regulatory Control of Cell Proliferation in Normal, Premalignant, and Malignant Colonic Tissues in Familial Polyposis
MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CP-02199)	Laboratory Service for Support in Carcinogenesis Bioassay and Related Activities
MIDDLESEX HOSPITAL MEDICAL SCHOOL (NO1-CP-75938)	Long-Term Studies of Prevention of Epithelial Cancer by Retinoids
MIDDLESEX HOSPITAL MEDICAL SCHOOL (NO1-CP-75955)	Studies of Carcinogenesis in Human Tissues
MIDWEST RESEARCH INSTITUTE (NO1-CP-75911)	Synthesis of Radioactive Retinoids for Metabolic and Pharmacologic Studies Relating to Prevention of Lung Cancer and Other Epithelial Cancers
MINNESOTA, UNIVERSITY OF (NO1-CP-55702)	Rhythmometry on Japanese and North American Female Volunteers of Different Age Groups
MINNESOTA, UNIVERSITY OF (NO1-CP-33364)	Polycyclic Hydrocarbon Metabolism in the Respiratory Tract
NEBRASKA, UNIVERSITY OF (NO1-CP-33289)	Chemical Carcinogen-Induced Noduligenesis and Tumorigenesis in Whole Mouse Mammary Gland Organ Culture
NEW ENGLAND NUCLEAR CORPORATION (NO1-CP-75937)	Synthesis of Radioactive Retinoids for Metabolic and Pharmacologic Studies Relating to Prevention of Lung Cancer and Other Epithelial Cancers
NEW YORK MEDICAL COLLEGE (NO1-CP-75949)	Studies of Metabolic Capacity in Intestinal Mucosa
NEW YORK UNIVERSITY (NO1-CP-33260)	Studies in Pulmonary Carcinogenesis

TABLE II (CONTINUED)

UNIT CONTRACT	TITLE
NORTH CAROLINA, UNIVERSITY OF (NO1-CP-75956)	Studies of Carcinogenesis in Human Tissues
NORTHWESTERN UNIVERSITY (NO1-CP-75876)	Histogenesis of Guinea Pig Pancreatic Adenocarcinoma
ORGANIZATION FOR HEALTH RESEARCH (TNO) (NO1-CP-33330)	Synergistic Interaction of Hormones and Neutron Radiation of Mammary Gland Carcinogenesis
PASADENA FOUNDATION FOR MEDICAL RESEARCH (NO1-CP-65850)	Primary Culture of Normal, Human Prostatic Epithelial Cells
RESEARCH TRIANGLE INSTITUTE (NO1-CP-75932)	Synthesis of New Retinoids for In Vitro Studies of Lung Cancer and Other Epithelial Cancers
SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (NO1-CP-75951)	Studies of Metabolic Capacity in Intestinal Mucosa
SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (NO1-CP-75950)	Studies of Metabolic Capacity in Intestinal Mucosa
SOUTHERN RESEARCH INSTITUTE (NO1-CP-75914)	Isolation, Identification and Culture of Epithelial Cell Types from the Colon of Experimental Animals
SOUTHERN RESEARCH INSTITUTE (NO1-CP-22064)	Organ Culture Assay of Vitamin A Analogs
SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION (NO1-CP-33379)	Studies of Hormonal Factors of the Human and Animal Prostate
STANFORD RESEARCH INSTITUTE (NO1-CP-75931)	Synthesis of New Retinoids for In Vitro Studies of Prevention of Lung Cancer and Other Epithelial Cancers
STANFORD RESEARCH INSTITUTE (NO1-CP-75936)	Synthesis of Radioactive Retinoids for Metabolic and Pharmacologic Studies Relating to Prevention of Lung Cancer and Other Epithelial Cancers

TABLE II (CONTINUED)

UNIT CONTRACT	TITLE
STATE UNIVERSITY OF NEW YORK (at Stony Brook) (N01-CP-33361)	Studies of Carcinogenesis in Organ Culture of Trachea and Bronchi
STOCKHOLM, UNIVERSITY OF (N01-CP-33363)	Studies on Polycyclic Hydrocarbon Metabolism in the Respiratory Tract
TEXAS, UNIVERSITY OF (N01-CP-33362)	Studies of Polycyclic Hydrocarbon Metabolism in the Respiratory Tract
TORONTO, UNIVERSITY OF (N01-CP-75879)	Biochemical and Morphologic Components of Hepatic Carcinogenesis
UTAH, UNIVERSITY OF (N01-CP-55709)	Quantitation of Physiological Reflux in Pancreatic Duct of Primates
VERMONT, UNIVERSITY OF (N01-CP-33360)	Studies of Carcinogenesis in Organ Culture in Trachea and Bronchi
VETERANS ADMINISTRATION HOSPITAL (Tampa, Florida) (Y01-CP-55625)	Autoradiographic Study of the Cellular Response of the Respiratory Tract in Chemical Carcinogenesis
VETERANS ADMINISTRATION HOSPITAL (Washington, DC) (Y01-CP-60204)	Studies on Normal, Premalignant and Malignant Respiratory Epithelium of Humans
VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY (N01-CP-55685)	Relationship of Fecal Neutral Sterols to Large Bowel Cancer Risk
VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY (N01-CP-33334)	Comparative Fecal Flora Studies
W. ALTON JONES CELL SCIENCE CENTER (N01-CP-65762)	In Vitro Cultivation of Normal, Prostatic Epithelial Cells
WISCONSIN, UNIVERSITY OF (N01-CP-75905)	Long-Term Studies of Prevention of Epithelial Cancer by Retinoids

TABLE II (CONTINUED)

UNIT CONTRACT	TITLE
2. CHEMISTRY OPERATIONAL UNIT	
ALBERT EINSTEIN COLLEGE OF MEDICINE (N01-CP-55606)	Studies on Microsomal Enzyme Systems Metabolizing Polycyclic Hydrocarbins in Experimental Animals and Humans
AMERICAN HEALTH FOUNDATION (N01-CP-55639)	Metabolism of Carcinogenic Compounds
BRITISH FOOD MANUFACTURING INDUSTRIES RESEARCH ASSOCIATION (N01-CP-44337)	Development and Application of Methods for N-Nitroso Compounds and Their Precursors in the Environment
CALIFORNIA, UNIVERSITY OF (San Diego) (N01-CP-33232)	Study on Pulmonary Tumors in Mice for Carcinogenic and Co-carcinogenic Bioassay
CALIFORNIA, UNIVERSITY OF (San Francisco) (N01-CP-43239)	Selection and Propagation of Somatic Cells Having Specific Physiological Mutations
DOE-NCI INTERAGENCY AGREEMENT (Brookhaven National Laboratory) (Y01-CP-50202)	Repair Mechanisms in Carcinogenesis
HAWAII, UNIVERSITY OF (N01-CP-75915)	Cycasin and Macrozamin as Potential Environmental Carcinogens
HEALTH RESEARCH, INC. (Roswell Park Memorial Institute) (N01-CP-55629)	Aryl Hydrocarbon Hydroxylase in Human Lymphocytes and the Relationship to Chemical Carcinogenesis
HEBREW UNIVERSITY (N01-CP-43307)	Mammalian Cell Transport
INSTITUTE OF CANCER RESEARCH (Chester Beatty Research Institute) (N01-CP-33367)	Nature of the Polycyclic Hydrocarbon-Nucleic Acid Compound in Hydrocarbon Carcinogenesis
JOHNS HOPKINS UNIVERSITY (N01-CP-55713)	The Significance to Mutagenesis in Carcinogenesis
JOHNS HOPKINS UNIVERSITY (N01-CP-45610)	Studies of Mammalian Cell Transport Systems

TABLE II (CONTINUED)

UNIT CONTRACT	TITLE
MASSACHUSETTS INSTITUTE OF TECHNOLOGY (N01-CP-43265)	Toxicity and Carcinogenicity Associated with Fungal Growth on Foodstuffs
MASSACHUSETTS INSTITUTE OF TECHNOLOGY (N01-CP-33238)	Interactions Between Diet and Chemical Carcinogenesis: A Bioassay System
MASSACHUSETTS INSTITUTE OF TECHNOLOGY (N01-CP-33315)	Environmental Occurrence of N-Nitroso Compounds
MISSISSIPPI, UNIVERSITY OF (N01-CP-43347)	Development and Application of Methods for N-Nitroso Compounds and Their Precursors in the Environment
MISSOURI, UNIVERSITY OF (N01-CP-75946)	Retroaldol Type Fragmentation of β-Hydroxy Nitrosamines
NEW YORK UNIVERSITY MEDICAL CENTER (N01-CP-33279)	Isolation, Propagation, and Storage of Mutant Vertebrate Cells with Specific Biochemical Lesions
ONTARIO CANCER INSTITUTE (N01-CP-43331)	Isolation, Propagation, and Storage of Mutant Vertebrate Cells with Specific Biochemical Lesions
PACIFIC NORTHWEST RESEARCH FOUNDATION (N01-CP-55719)	Metabolism of Carcinogenic Compounds
ROCHESTER, UNIVERSITY OF (N01-CP-45611)	Mammalian Cell Transport Systems
SOUTHERN RESEARCH INSTITUTE (N01-CP-55721)	Metabolism of Carcinogenic Compounds
TEXAS, UNIVERSITY OF (Houston) (N01-CP-55604)	Studies of Microsomal Enzyme Systems Metabolizing Polycyclic Hydrocarbons in Experimental Animals and Humans
VANDERBILT UNIVERSITY (N01-CP-65730)	Non-Histone DNA-Binding Proteins in Chemical Carcinogenesis
WEIZMANN INSTITUTE OF SCIENCE (N01-CP-02217)	Study of the Role of Enzyme Induction in Chemical Carcinogenesis
WEIZMANN INSTITUTE OF SCIENCE (N01-CP-33220)	Studies of Alterations Produced by Chemical Carcinogens in Viral and Cellular Gene Expressions

TABLE II (CONTINUED)

UNIT CONTRACT	TITLE
WISCONSIN CLINICAL CANCER CENTER (N01-CP-65734)	Induction of Aryl Hydrocarbon Hydroxylase in Cultured Human Lymphocytes
WISTAR INSTITUTE (N01-CP-55655)	Significance of Mutagenesis in Carcinogenesis
YALE UNIVERSITY (N01-CP-55673)	Selection and Propagation of Monosomic and Haploid Cell Lines

3. INTERDISCIPLINARY PROGRAMS AND RESOURCES OPERATIONAL UNIT

UNIT CONTRACT	TITLE
CHICAGO, UNIVERSITY OF (N01-CP-33385)	Synthesis of Derivatives of Carcinogenic Polycyclic Hydrocarbons
CHILDREN'S HOSPITAL OF PHILADELPHIA (N01-CP-65803)	Oncogenesis and Other Late Effects of Cancer Therapy
COLUMBIA UNIVERSITY (N01-CP-23234)	Development of a Tissue Culture Transformation System for Aromatic Amine Carcinogens
DOE-NCI INTERAGENCY AGREEMENT (Oak Ridge National Laboratory) (Y01-CP-50200)	NCI-DOE Carcinogenesis Program
FRANKLIN INSTITUTE RESEARCH LABORATORIES (N01-CP-65812)	Information Support: Retinoid Program/ Human Tissue Study Program
HARLAN INDUSTRIES, INC. (N01-CP-55647)	Development of Colonies of Aged Rats
IIT RESEARCH INSTITUTE (N01-CP-65745)	Preparation of Carcinogens
IIT RESEARCH INSTITUTE (N01-CP-55646)	Chemical Repository
INFORMATION PLANNING ASSOCIATES, INC. (N01-CP-75896)	Management and Technical Support Services to the Carcinogenesis Research Program
MIDWEST RESEARCH INSTITUTE (N01-CP-65739)	Preparation of Carcinogens

TABLE II (CONTINUED)

UNIT CONTRACT	TITLE
MIDWEST RESEARCH INSTITUTE (N01-CP-23270)	Analytical Chemistry Resource
MIDWEST RESEARCH INSTITUTE (N01-CP-33387)	Synthesis of Polycyclic Hydrocarbon Derivatives
NCI FREDERICK CANCER RESEARCH CENTER (Litton Bionetics, Inc.) (N01-CO-75380)	Operation and Maintenance of the Frederick Cancer Research Center
NEBRASKA, UNIVERSITY OF (Eppley Institute for Research in Cancer) (N01-CP-33278)	A Resource for Carcinogenesis Bioassays and Related Research
NEW HAMPSHIRE, UNIVERSITY OF (N01-CP-55675)	Preparation of Various N-Nitroso Compounds
SALK INSTITUTE FOR BIOLOGICAL STUDIES (N01-CP-75970)	Chemical Carcinogenesis and Control of the Cell Cycle
SOUTHERN RESEARCH INSTITUTE (N01-CP-65740)	Preparation of Carcinogens
STANFORD RESEARCH INSTITUTE (N01-CP-65741)	Preparation of Carcinogens

5. PROGRAM SUMMARY REPORTS

SUMMARY REPORT

BIOLOGY BRANCH

October 1, 1977 through September 30, 1978

"Plans, develops, and conducts a research program aimed at (1) elucidation of the role of chemical, physical and biological agents in the process of carcinogenesis, (2) characterization of cellular alterations associated with carcinogenesis, (3) evaluation of the relationships between mutagenesis and carcinogenesis, and (4) the development and definition of in vitro cellular transformation systems for the rapid detection of the carcinogenic actions of chemicals."

The Biology Branch has continued to develop mammalian cell in vitro carcinogenesis models for the rapid identification of cancer causing agents relevant to the human environment, and the study of events and mechanisms that accompany and/or cause malignancy. Methods to prevent and reverse the carcinogenic process are also being investigated. Three aspects of the carcinogenic process are under study--(1) transformation of normal to neoplastic cells, (2) immunobiologic evaluation of the neoplastic state, and (3) relationship of mutational events to the transformation. The major research projects in the Biology Branch continue to be--(1) development of short-term and reliable methods, based on in vitro cell transformation, to identify carcinogenic chemicals, (2) development of methods to identify and characterize the stages of carcinogenesis, and (3) assessment of the relationship of somatic cell alterations to the neoplastic state. Responsibility for these projects was divided between two sections: Somatic Cell Genetics Section and Tumor Biology Section. Results of studies from the individual sections were coordinated at the branch level to determine their applicability to the goals of the National Cancer Program. In the Somatic Cell Genetics Section, studies concerning the modulation of transformation frequencies by sequential treatment with different agents has continued. With multiple agents, the transformation data is consistent with a one-hit hypothesis; however, the same multiple agents do not increase the mutant frequency of the gene locus examined. Studies have been expanded to include human cells which can be transformed by direct acting carcinogens, the adaptation of the Syrian hamster colony analysis to a reproducible focus assay, and the use of sister chromatid exchanges for identifying carcinogens as well as for studying chromosomal changes. Members of the Tumor Biology Section have continued to study specific stages in the carcinogenic process identifiable during chemical carcinogen induced neoplastic transformation of guinea pig cells in culture in order to provide valuable insights into the events that occur during development of a cancer cell. Guinea pig cells are resistant to lymphotoxin during various stages of carcinogenesis prior to expression of tumor growth; susceptibility, the inhibition of tumor cell growth, develops in close proximity or concomitantly with neoplastic transformation.

Somatic Cell Genetics Section - "*Studies (1) carcinogenesis in mammalian cells in vitro and in host-mediated in vivo and in vitro models, (2) interactions of chemical, physical and biological agents resulting in the initiation, enhancement or inhibition of neoplastic transformation, (3) effects of potential environmental carcinogens in cells in culture and in intact animals, and (4) relationships between mutagenesis and carcinogenesis in in vitro mammalian cell models and in intact animals.*" The prevention of cancer in humans depends to a large extent on finding and removing potentially harmful environmental agents. The use of mammalian cells in culture is particularly appropriate because they represent an extension of experimental animal models. The conversion of control mammalian cells to the neoplastic state in culture can be validated by injecting the cells into animals to produce progressively growing tumors which kill the animals.

In vitro quantitative models utilizing mammalian cells have continued to be the basis for studying the fundamental steps that lead to transformation and for determining the potential carcinogenicity of certain agents suspected of being deleterious to humans. Relatively short-term cultures are being used to study the relationship of DNA damage/repair to transformation, and the relationship of mutagenesis, at both the gene and chromosome level to transformation. The results with cells of different species are being compared to determine metabolic differences in the mode of action of various agents that lead to transformation. In addition, new models are being devised that will be useful as probes for identifying potential carcinogens.

Significant data has been obtained which indicates a dose-response relationship and a zero threshold level with different carcinogens. Some carcinogens negative by direct application are positive when tested in a transplacental host-mediated assay. Under these circumstances, vinyl chloride is a transforming agent whereas it fails to have an effect when applied directly to cells. The host-mediated assay appears to be a powerful tool that should be included in the armament of environmentalists interested in determining potential carcinogenicity of materials in the human environment.

The Syrian hamster colony model was also modified so that the transformed foci could be scored on a background of normal cells. The foci morphology is verified by steroscropic examination and consequently the number of areas that need to be examined is greatly reduced compared to the colony assay. The frequency of foci formation relative to different chemical concentrations was independent of the cell number within a certain range but was dependent upon chemical concentration, length of treatment, and time of the chemical addition after cell transfer. The foci could be isolated after about three weeks growth and their tumorigenicity verified by transplantation into weanling hamsters. The frequency of transformation can be related to the lethality by determining cloning efficiency, again without a feeder layer. The reliability of the system was established by obtaining reproducible results in two geographically separated laboratories, in Chicago and in Bethesda. Since the transformation can be isolated and shown to produce tumors when the cells are injected into animals, transformation is the most relevant approach for determining carcinogenicity.

The use of primary cell cultures derived from human tissue is of interest. Whereas a number of environmental carcinogens will transform cells *in vitro* derived from animals of different species, reports of putative transformation of human cells *in vitro* have been sporatic. A review indicates that human cells are very efficient repairers of DNA damage. Thus, the possibility exists that the deleterious effects of chemical carcinogens on the cells are quickly eliminated. When primary human cells were arrested in the G_1/S interphase of the cell cycle, released from the block, and treated with insulin to insure the optimum number of cells in the S phase before treatment with a carcinogen for approximately 10 hrs, a number of morphologically altered cells were seen in the culture. Over 20 lines have been established from cells grown previously in agar and the majority of these have been shown to produce mesenchymal tumors in nude mice. All cell lines have a human chromosomal constitution with a near diploid stemline; however, despite the normal diploid chromosome number, G-band analysis has demonstrated that these cell lines are pseudo-diploid and contain numerical deviations or abnormal chromosomes. The heterochromatin distribution was normal, as was reported several years ago for transformed Syrian hamster cells. The detailed heterochromatin analysis did reveal alterations such as pericentric inversions in chromosomes with large areas of heterochromatin or abnormal chromosomes with more than one centromere.

Mutagenesis is suspected of being the mechanism for carcinogenesis. There is no question that DNA alterations result in mutation. The underlying mechanism of transformation that leads to carcinogenesis by chemical and physical agents may also involve mutation. The relationship between malignant transformation and mutagenesis requires reliable measurements of induced mutation frequencies in different cell types. Our procedures result in obtaining reliable mutation frequencies under optimal conditions. For example, although caffeine is an inhibitor of post-replication repair of DNA in Chinese hamster V79 cells and usually reduces cell survival after a mutagen, the frequency of azaguanine or thioguanine resistant cells in a surviving population is definitely unchanged by the action of caffeine. On the other hand, transformation by a chemical carcinogen is enhanced by post-treatment with caffeine. The increase in transformation occurs without an increase in toxicity. Combinations of agents have produced co-carcinogenesis, syncarcinogenesis, or enhancement of transformation *in vitro*. Synergism of mutation frequency of two or more agents in cultured mammalian cells has not been reported. Chinese V79 cells were treated with a weak and a potent carcinogen and the induced mutation frequency determined. Lethal synergism was observed for the two treatments, but the mutant frequency was unaltered. These results emphasize the difficulties in extrapolating from one species to another.

Cultured human fibroblasts from normal individuals and Xeroderma pigmentosum patients may be used to determine the roles of DNA repair defects in mutagenesis and carcinogenesis caused by ultraviolet irradiation. The mutant frequency for animal and human cells has varied from 10^{-5} to 10^{-7}/cell/erg/mm^2 while the transformation frequency is approximately 10^{-3}/cell/erg/mm^2. Ultraviolet mutagenesis of normal human cells has indicated that the expression time for UV-induced thioguanine resistant phenotypes is about 10-12 days. By using stringent selection conditions and avoiding metabolic crossfeeding during selection, the real maximum induced frequency is one tenth the previously reported frequencies for human cells. Selection was performed with

20 μg/ml thioguanine in medium containing fetal bovine serum, which allows approximately 80% survival of Lesch-Nyhan (mutant prototype) cells and completely eliminates all normal, nonmutant cells.

The relationship of chromosomal changes to neoplasia is one of the most controversial subjects in cancer research. Chemical, physical, and biological carcinogens induce chromosomal damage after a short exposure in cell culture. This damage, upon the species or agent utilized, may randomly affect various portions of the genome or, may be restricted to a specific part of the genome. Recent cytological methods for detecting sister chromatid exchanges (SCE) with bromodeoxyuridine (BrdU) and fluorescence or Giemsa staining have shown that SCE is a phenomemon inducible by a large number of mutagens/carcinogens and indicate that SCE are sensitive indicators of chromosome damage repair. All direct acting carcinogens increased the frequency of SCE in Chinese hamster cells. Noncarcinogens were ineffective while indirect acting carcinogens increased SCE only in a cell-mediated system which permitted metabolic activation. It has been postulated that SCE are a result of post-replication DNA repair. Experiments with caffeine, a known inhibitor of post-replication repair, has shown that post-treatment with caffeine enhances the frequency of chromosomal aberrations induced by agents as alkylating agents or ultraviolet irradiation if present during the "S" phase of the cell cycle. Similarly, post-treatment with caffeine enhances SCE only after UV, X-ray, 4-Nitroquinoline-1-oxide, and methyl methanesulfonate.

One protocol for the treatment of psoriasis involves a clinical photo-chemotherapy regime designated PUVA, a combination of 8-mythoxysoralen (8-MOP) and long wavelength ultraviolet light (UVA). The combination leads to mono- and biofunctional psoralen-DNA photoadducts. The addition of calculated therapeutic dose levels of 8-MOP and ultraviolet light will double the number of SCE's in a human lymphoblastoid cell line whereas neither chemical nor UVA influences the SCE frequency. An important question now arises whether patients also develop a sensitivity to the photochemotherapy as indicated by an increase in SCE.

Lastly, the indepth cytogenetic analysis of Syrian hamster chromosomes constitution has been continued because of the relevance of Syrian hamster cells to neoplastic transformation. Additional information concerning heterochromatin characteristics of Syrian hamster cells was obtained by new methods for late replication by BrdU substitution and Giemsa stain. A new karyotype on the basis of heterochromatin was established. By use of denaturing agents such as sodium hydroxide or barium hydroxide, the autosomal centromeric heterochromatin was differentiated from the heterochromatin that composes the short arms of the autosomes. The genus Mesocrietus has, in addition to the Syrian hamster, two other species; m. newtoni and m. brandti. Consideration of G- and C-band analysis leads to the deduction that the golden hamster is probably the ancestral species of this particular genus.

Tumor Biology Section - *"Studies somatic cell alterations associated with the transition from the growth controlled to the neoplastic state to define intracellular and extracellular events at the cellular and host levels essential for the development, maintenance and/or suppression of neoplasia."* The major focus of investigative activities continues to be the definition of the pheno-

typic alterations associated with the stages of carcinogenesis. The chemical carcinogen induced transition from the growth controlled to the neoplastic state of cultured fetal guinea pig cells is characterized by multiple sequential stages. These extend from normal morphologic orientation, progress through preneoplastic morphologic alteration, preneoplastic morphologic transformation, and culminate in neoplastic morphological transformation. Identification of these persistent stages which often span months of subculture in this *in vitro* carcinogenesis model was initially observed in cells from freshly cultured, carcinogen-treated fibroblast-like cells. Current investigations with a continuous nontumorigenic fibroblast-like guinea pig cell line demonstrate the identical sequences. Thus, carcinogenesis can occur independent of the age and number of cell divisions of the target cells at the time of carcinogen exposure. More specifically, the frequency of carcinogen induced transformation, latent period between carcinogen exposure and development of neoplastic transformation, and the multi-stage nature of the transition are similar in freshly cultured and long-term continuously passaged guinea pig cells. Some somatic cell alterations such as susceptibility to the cytotoxic activity of lymphotoxin and the ability to form colonies in soft agar usually develop concomitantly with the onset or during the stage of morphological transformation. Lymphotoxin susceptibility may be expressed simultaneously with, before or following, colony formation in agar. In addition, either or both phenotypic alterations can develop many cell generations and months before conversion of the morphologically transformed preneoplastic (nontumorigenic) target cell to the neoplastically transformed state. Another carcinogen induced phenotypic alteration is the production of a positive intradermal skin test in nonimmune animals. Unlike colony formation in agar and lymphotoxin susceptibility, natural skin reactivity has been observed only with morphologically transformed cells that are capable of producing tumors. Continuing evaluations are aimed at defining the mechanisms of lymphotoxin susceptibility and natural skin reactivity; both are distinct phenotypic alterations associated with different stages in the transition from the growth controlled to the neoplastic state and present potential points for immunobiological modulation of *in vivo* carcinogenesis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04575-04 BGY

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Relationships Between In Vitro Mutagenesis and Chemical Carcinogenesis.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. C. Myhr	Senior Staff Fellow	BGY NCI
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	D. Turnbull	Visiting Fellow	BGY NCI

COOPERATING UNITS (if any)
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LAB/BRANCH
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.3	1.8	0.5

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
It is the long range purpose of this project to determine whether mutational processes are involved in in vitro malignant transformation of mammalian cells by diverse chemical and physical carcinogens. Research efforts during the past year have centered on (1) determination of cell culture conditions necessary to maintain morphological differences between transformed and normal cells, (2) standardization of the survival responses of Chinese hamster V79 cells to chemical carcinogens, caffeine, and UV light, and (3) study of assay conditions necessary for quantitative measurements of induced mutation frequencies in cultured X. pigmentosum human cells. Future efforts will be directed toward simultaneous mutagenesis and transformation assays utilizing both human and Syrian hamster embryo cells in culture.

PHS-6040

Project No. Z01 CP 04575-04 BGY

Project Description

Objectives: The overall objective of this project is to determine whether mutational processes are involved in the neoplastic transformation of mammalian cells by diverse chemical and physical mutagens. The accomplishment of this goal depends on reliable quantitation of induced mutant frequencies using different types of cultured cells. The immediate objectives are:

1. To establish the mutagenic and survival responses of V79 Chinese hamster cells to sequential chemical treatments that are known to cause enhancement of neoplastic transformation.

2. To establish and validate a quantitative assay for induced mutant frequencies in normal human and X. pigmentosum cell cultures.

3. To determine whether the concentration and choice of selective agent for purine analog-resistant mutants of different cell types can be based on early metabolic responses and the survival of mutant prototypes.

Methods Employed: Standard cell culture procedures were used to determine cloning efficiencies and survival to toxic treatments as measured by colony-forming ability. Mutagenesis assays were based on resistance to 8-azaguanine (AZG) or 6-thioguanine (TG) and were carried out according to procedures developed by this laboratory. Results of other studies have been greatly distorted by crossfeeding between mutants and non-mutants in treated colonies of cells and/or by lack of sufficient expression times. For quantitative studies, cells are reseeded at various times following mutagenic treatment to permit maximum expression of the resistant phenotype before selection and to avoid metabolic crossfeeding by wildtype cells. The attainment of maximum induced mutation frequencies is essential for the determination of dose-response relationships and the effects of DNA repair-modifying agents or comparing the responses of normal and DNA repair-deficient cells. The resistant phenotype is based upon reduced levels of hypoxanthine guanine phosphoribosyl transferase (HGPRT) and this activity was measured in situ in normal and Lesch-Nyhan (HGPRT-deficient) human cells by labeling with ^{14}C-hypoxanthine or ^{14}C-guanine in growth medium. Phosphorylated products of the enzyme reaction were selectively adsorbed to DEAE-paper discs and counted. Adenine phosphoribosyl transferase (APRT) activity was similarly determined by using ^{14}C-adenine labeling.

Major Findings: 1. Caffeine is an inhibitor of post-replication repair of DNA in Chinese hamster V79 cells, and this activity is reflected in the greatly reduced survival of V79 cells exposed to a mutagen, such as N-acetoxy-2-acetylaminofluorene (AcAAF), in the presence of caffeine. In undamaged cells, caffeine up to 1 mM does not impair cell survival. Previously we showed that the frequency of AZG- or TG-resistant cells in the surviving population is unchanged by the action of caffeine. Therefore, caffeine appears to reduce the efficiency of DNA repair, resulting in lower cell survival, without altering the probability of error in the repair that does take place. Studies were continued to determine the specific relationships between the induced

mutant frequency and the toxicity of treatment. Since AcAAF is highly reactive and caffeine is reported to be rapidly metabolized by V79 cells, survival must be determined at chemical-to-cell ratios that are used during mutagenesis. The treatment of about 10^6 cells is necessary for mutagenesis experiments, while survival studies are normally performed with 10^3 - 10^4 viable cells. By mixing X-irradiated, non-proliferating V79 cells with viable cells, it was established that the survival to a given treatment of AcAAF alone or in combination with caffeine begins to increase at 10^5 cells and may become 10-fold greater by 2 X 10^6 cells. This increased survival appears to be the result of less AcAAF availability per cell rather than the depletion of caffeine by the larger number of cells. Caffeine depletion in the growth medium was estimated by using standard survival curves. Less than one-fourth of 1 mM caffeine was metabolized in the first several hours by 10^6 cells and then a toxic product(s) was released that by 48 hr metabolism reduced the survival below that of fresh 1 mM caffeine in the assay. In contrast, X-rayed cells did not metabolize caffeine as quickly and did not produce detectable amounts of toxic metabolites.

2. Synergism between the mutagenic activities of two or more agents in cultured mammalian cells has not been reported, although enhancement of lethality and transformation frequencies have been found for certain combinations. Treatment of V79 cells with 500 μM methyl methanesulfonate (MMS) for 1 hr resulted in 95% survival of cloning ability and induced a mutant frequency of 4 X 10^{-5}. 7 μM AcAAF produced 10 times as many mutants and the survival was 7%. Lethal synergism was observed for the MMS treatments followed by 7 μM AcAAF, and the degree of synergism was unaffected by increasing the time interval between treatments from 1 to 48 hr. However, no significant changes in the mutant frequency from that induced by AcAAF alone was found for treatment intervals of 1 to 63 hr. This result with V79 cells contrasted with the 6-fold enhancement of AcAAF-induced transformation of Syrian hamster embryo cells exposed to an equivalent combination with a 48 hr interval between treatments.

3. Lesch-Nyhan (L-N) human fibroblasts are deficient in HGPRT and therefore are useful in the design assay conditions for quantitation of induced $HGPRT^-$ phenotypes in normal human cell cultures. The survival of different L-N strains of AZG and TG was determined; all strains were similar and showed much greater resistance to TG than to AZG in growth medium containing dialyzed fetal calf serum (FBS). With undialyzed FBS, the survival to AZG was nearly the same as for TG. The results indicate that purines in the serum compete very effectively with the AZG (but not TG), and in the absence of purine competition AZG is very toxic to L-N cells (compared to TG). The basis for the toxicity appears to be the low levels of HGPRT activity (6% or higher) associated with L-N cells. Mutation assays designed to detect cells with L-N-like HGPRT activities could therefore use 1 μg/ml AZG or 20 μg/ml TG, in medium containing dialyzed FBS for about 80% survival of induced mutants. L-N survival drops sharply with higher AZG concentration. Thus, incorporation of small amounts of AZG appears far more toxic to human cells than the incorporation of similar amounts of TG.

4. Human diploid cells have a propensity for cell-to-cell contacts and therefore must be seeded at very low densities (5-10 cells/mm^2) to avoid metabolic

crossfeeding during selection for induced AZG or TG-resistant cells. The recovery of mutant cells from wildtype populations might be increased or made possible at higher cell densities by choosing selection media in which the synthesis of purine analog phosphorylated derivatives is reduced as quickly as possible. Since inosine monophosphate production in the presence of AZG or TG is complicated by competition between labeled hypoxanthine and the analogs, the analogous and independent pathway of adenosine monophosphate (AMP) production from exogenously supplied labeled adenine was used. The results showed that loss of wildtype enzyme is slow during exposure to TG. Cell division occurs at 2 μg/ml TG and wildtype enzyme activity actually increases over a 4-day exposure period. At 20 μg/ml TG, wildtype AMP synthesis remains nearly unchanged over 4 days exposure and decreases after 7 days. Selection of a mutagenized population of human cells with 2 μg/ml TG yields a heavy background of wildtype cells and a low frequency of resistant colonies; at 20 μg/ml TG, wildtype cells are not observed and the frequency of resistant colonies is higher.

AZG acts much more rapidly than TG in reducing AMP production by wildtype cells. At 3 μg/ml AZG, the enzyme activity is reduced to 30% by 2 days exposure. Higher concentrations of AZG are not much more effective. As noted earlier, however, AZG becomes highly toxic to L-N cells above 1 μg/ml in growth medium containing dialyzed FBS (no purine competition). Therefore, selection with AZG at 1 μg/ml in medium containing dialyzed FBS should be about equivalent in selection pressure against wildtype to 20-30 μg/ml TG in whole FBS. The AZG selection has the disadvantages, however, of poorer cloning efficiency and growth in dialyzed FBS and a sharp dependence on small changes in AZG concentration. AZG decomposes in growth medium and must be replaced at frequent intervals.

5. Ultraviolet (UV) mutagenesis studies of normal human cells have yielded several important observations. The reseeding procedure for mutant selection, as initiated by this laboratory, appears necessary for the detection of TG-resistant colonies. The time necessary for the expression of UV-induced TG-resistant phenotypes is much longer than previously thought, requiring about 10-12 days. The maximum UV-induced mutant frequency determined with 20 μg/ml TG is also about one-tenth the frequency in previous reports where 3 μg/ml AZG was used in medium containing whole calf serum (unknown amounts of competing purines).

Significance to Biomedical Research and the Program of the Institute: Mutagenesis has long been a suspected, but undemonstrated, mechanism for chemical and physical carcinogenesis. Since most human cancers are thought to be caused by environmental agents, it becomes extremely important to understand the involvement of DNA alterations caused by these agents. The survival and mutagenesis studies with Chinese hamster V79 cells have clearly shown that cultured mammalian cell lines in general will not necessarily be useful models for determining the mechanisms of neoplastic transformation of normal human cells. DNA repair processes are central to the production of viable mutants (and possibly transformants) and these processes may differ greatly in kind, amount, and in proneness to error among different cell types. Thus, continued efforts to validate mutagenesis in non-malignant human cells and other normal

cell types in culture are crucial to understanding the involvement of mutation in neoplastic transformation.

Proposed Course of the Project: Mutagenesis studies of normal human cells and *X. pigmentosium* cell types will be continued in order to determine the involvement of different DNA repair pathways. UV light and activated chemical carcinogens will be used. The genetic markers will be TG-resistance and ouabain resistance. The quantitation of mutagenesis in Syrian hamster embryo cell cultures and in normal and transformed guinea pig cell lines will also be studied in order to relate mutation and transformation in the same cell type.

Publications

Myhr, B. C., and DiPaolo, J. A.: Mutagenesis by N-acetoxy-2-acetyl aminofluorene of Chinese hamster V79 cells is unaffected by caffeine. *Chem.-Biol. Interact.* (In Press).

Myhr, B. C., and DiPaolo, J. A.: Comparison of mutagenesis and malignant transformation *in vitro* with methyl methanesulfonate and N-acetoxy-2-acetyl aminofluorene. *Cancer Res.* (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04629-13 BGY

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

The Mechanism of Cell Transformation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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3.5	2.5	1.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The conditions which are responsible for or can alter in vitro transformation will be investigated further, with specific emphasis on the use of UV, X-rays, combinations of carcinogens of different classes, the combination of in vivo - in vitro systems, and the effect of stimulators and inhibitors of mixed function oxidases on toxicity and transformation. Studies on the mechanisms of early events of carcinogen-cell interaction will be continued, as well as investigation of different proximate carcinogens and metabolic inhibitors. Special emphasis will be given to the requirements necessary to obtain neoplastic transformation of human fetal cells. The testing for the tumorigenicity of cells exposed to different agents for different time intervals will continue.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: The primary objective of this project is to establish conditions and methods for *in vitro* quantitative study of chemical transformation. The immediate targets of study are:

1. Development of rapid assay systems for chemical carcinogens suitable for the screening of compounds and populations.

2. Define the conditions required for the transformation of human fetal cells.

3. Determine ways to increase the susceptibility of primary cell lines or cell strains to chemical transformation.

4. Development of assays to determine whether *in vitro* transformation is accompanied by the appearance of tumor specific antigens.

5. Determine the role of nononcogenic and oncogenic viruses in chemical transformation.

6. Determination of the ultrastructural changes of cells during the course of *in vitro* chemical transformation so that they may be contrasted with normal cells and with information pertaining to viral transformation.

7. Determine the role of repair, e.g. rate, excision and post-replication in enhancing neoplastic transformation with radiation and alkylating compounds.

8. The transformation of epithelial-like cells which result in the formation of carcinomas when the cells are transplanted into animals.

9. Analysis of variations in populations of somatic cells and their correlation with genetic changes.

Methods Employed: All procedures performed are with the view of quantitating phenomena *in vitro*. Such procedures are required to determine whether or not the transformation observed is due to the direct or indirect effect of the carcinogen and in order to study the early events associated with *in vitro* transformation. Cells used come from freshly isolated cells from animals and humans that as controls have many of the attributes of "normal" cells and from cell lines which are known to exhibit some of the properties associated with nontransformed cells. Discrete cells are grown in complete medium in the presence or absence of irradiated rat or hamster cells. The cells may be derived from whole embryos or may be from specific organs. The cells are exposed to chemical carcinogen transplacentally or prior to or subsequent to seeding the cells in Petri dishes.

Approximately one week subsequent to treatment, the cells are examined under phase or under stained conditions for number of transformed colonies, toxicity, and spectrum of morphology of both the normal and transformed colonies. The frequency of transformation is expressed in a number of different ways. These

take into consideration the observed rate of transformation on a per-cell basis or on a number of colonies obtained.

In vitro quantitative models utilizing mammalian cells have continued to be the basis for studying the fundamental steps that lead to transformation and for determining the potential carcinogenicity of certain agents that are suspected of being deleterious to humans. In the latter case, significant data has been obtained which indicates a dose-response relationship and a zero threshold level. The transformation frequency when plotted relative to carcinogen concentration results in a curve with a slope of approximately one on a semi-log basis. As a result, it has been concluded that transformation is consistent with the one-hit hypothesis. Mathematical models and statistical methodology have been applied to the interpretation of cell transformation data in hamster embryo cells. Under the assumption of binomial variation, the one-hit curve has been shown to fit several sets of experimental data. Maximum likelihood estimation methods have been used with two statistical tests of fit. Enhancement of the effect of irradiation on chemical transformation was quantified by the ratios of the parameters of the fitted one-hit curves and standard errors of this measure were developed. Statistical examination of randomness was applied to the distribution of the numbers of the transformed cells among the dishes. In addition, the total number of cells at risk was estimated using statistical methods developed for use with the number of dishes in an experiment. This statistical analysis constitutes conclusive proof that transformation is an inductive phenomenon.

Evidence exists that a number of inorganic metals can cause a variety of cancers in in vivo experimental models. Furthermore, there is epidemiological indication that some inorganic metals are carcinogenic in humans. Currently, 15 inorganic metals have been tested with the quantitative Syrian hamster embryo cell system. Transformation was found for eight metals, including two nickels, two chromates, cadmium, arsenite, beryllium, and lead. The negative compounds were iron, titanium, molybenate, zinc, aluminum, and amorphous nickel.

The metals capable of transforming cells are also able to enhance the transformation ordinarilly associated with a DNA virus. The significance of these results is that the transforming metals are related to the work environment of a number of different industries. These metal carcinogens cannot be identified with popular microbial systems. The molecular events associated with transformation by the metals is probably related to the breaks produced by the metals which affect the fidelity of DNA synthesis. The relationship of transformation of DNA repair and DNA break was determined with 50 chemicals; whereas transformation identified accurately 90% of the chemicals, DNA repair and DNA fragmentation identified only 50 and 70% of the chemicals, respectively.

The use of primary cell strains relative to the use of cell lines have advantages but primary strains have relatively low cloning efficiencies, require the use of feeder layers and ideal growth conditions. The Syrian hamster embryo model was modified so that the transformed foci could be scored on a background of normal cells. The foci morphology is verified by steroscopic examination and consequently the number of areas that need to be examined is

greatly reduced compared to the colony assay. The frequency of foci formation relative to different chemical concentrations was independent of the cell number within a certain range but was dependent upon chemical concentration, length of treatment, and time of the chemical addition after cell transfer. The foci could be isolated after about three weeks growth and their tumorigenicity verified by transplantation into weanling hamsters. The frequency of transformation can be related to the lethality by determining cloning efficiency, again without a feeder layer. The reliability of the system was established by obtaining reproducible results in two geographically separated laboratories, in Chicago and in Bethesda. Thus far the method which has been developed with known positive and negative chemical carcinogens has shown no false positives or negatives.

On ocassion, certain chemicals which require metabolic activation of the type which cannot be carried out by cells in culture will give false results. This was circumvented by the transplacental host-mediated assay which we reported several years ago. In this assay, the dam is given the suspected chemical interpertonatally approximately 48 hrs before the fetuses are removed. The cells are prepared as usual and the transformation is readily apparent approximately 2 passages later. This protocol was applied to vinyl chloride monomer. Under these circumstances, vinyl chloride is a transforming agent whereas it fails to have an effect when applied directly to cells. This data is consistent with that obtained on bacteria and with yeast which required purified liver microsome fraction to convert the compound into the active metabolite which produced mutations. This host-mediated assay appears to be a powerful tool that should be included in the armament of environmentalists interested in determining potential carcinogenicity of materials in the human environment.

The use of primary cell cultures derived from human tissue is of interest. Whereas a number of environmental carcinogens will transform cells _in vitro_ derived from animals of different species, reports of putative transformation of human cells _in vitro_ have been sporatic. A review indicates that human cells are very efficient repairers of DNA damage. Thus, the possibility exists that the deleterious effects of chemical carcinogens on the cells are quickly eliminated. When primary human cells were arrested in the G_1/S interphase of the cell cycle, released from the block, and treated with insulin to insure the optimum number of cells in the S phase before treatment with a carcinogen for approximately 10 hrs, a number of morphologically altered cells were seen in the culture. After several additional population doublings, cultures grew more rapidly and at the approximate 20th population doubling, these cells were able to form colonies in agar. Over 20 lines have been established from cells that have formed colonies; the majority of these produce tumors when inoculated subcutaneously into nude mice. These mesenchymal tumors can be subpassaged into other animals three times. Currently, the treated lines are at the 110th population doubling. Control cultures fail to grow beyond 35 passages, form colonies in agar or to produce tumors.

Human fibroblasts transformed _in vitro_ with chemical carcinogens were analyzed for their chromosomal constitution. G and C-band techniques as well as Ag-As staining method for nucleolar organizer (NOR) were applied to nine individual

cell lines resulting from transformation with B-propiolactone, aflatoxin B_1, 4-nitroquinoline-1-oxide, ethyl methanesulphonate, propane sultone, and N-methyl-N'-nitro-N-nitrosoguanidine. All lines examined have a human chromosomal constitution with a near diploid stem line. G-band analysis demonstrated that some lines, despite their normal diploid chromosome number, are pseudodiploid, containing numerical deviations or abnormal chromosomes. No changes in the distribution of heterochromatin were found. However, C-band analysis revealed alterations such as pericentric inversions in chromosomes with large areas of heterochromatin or abnormal chromosomes having more than one centromere. This study will determine the significane of the chromosomal changes in the process of human cell transformation by chemical carcinogens.

It has been suggested that carcinomas, leukemias and sarcomas in rats induced by 7,12-dimethylbenz(a)anthracene (DMBA) are associated with specific changes in numbers of chromosome No. 2. Our transformation data did not support this conclusion since transformation or tumor induction in vivo was not accompanied specifically by alterations in chromosome No. 2. Recently rat embryo secondary cultures were treated with DMBA for 5, 9, 24 hrs and chromatid type of aberrations were found. In terms of incidence of chromatid lesions, chromosome No. 2 was the most susceptible autosomes. Banding pattern analysis demonstrated that the region associated with negative band 2q24 of chromosome No. 2 had the highest number of lesions. An increased accumulation of DMBA-^{3}H label also occurred in approximately the same chromatid area of a small fraction of cells exposed for either 5 or 9 hrs prior to mitosis. The complete loss of DMBA-^{3}H chromosomal labelling after DNAse treatment suggests that the visible grains represent carcinogen-bound DNA. After DMBA and 5-bromodeoxyuricin (BrdU), the number of sister chromatid exchanges (SCE) increased compared to controls treated with BrdU only; the location of the exchange points on chromosome No. 2 was also similar when treated with either DMBA and BrdU or BrdU alone. These studies demonstrate that a specific chromosome may be affected by diverse agents, that chromatid lesions frequently occur at the site of SCE and that they probably are not determinants of malignant development.

The relationship of chromosomal changes to neoplasia is one of the most controversial subjects in cancer research. Most carcinogenic agents, chemical, physical, or biological, induce chromosomal damage after a short exposure in cell culture. This damage may randomly affect various portions of the genome or, depending upon the species or agent utilized, may be restricted to a specific part of the genome. Recent cytological methods for detecting SCE with BrdU and fluorescence or Giemsa staining have shown that SCE is a phenomenon inducible by a large number of mutagens/carcinogens and indicate that SCE are sensitive indicators of chromosome damage repair.

Mitotic abnormalities often affect the centrioles. New cytochemical techniques permit the cytological identification of the centrioles and of nucleolar organizer associated regions. The latter are especially import for tumor cells with polyploid stemlines or chromosomes preferentially involved in centromeric association. These new cytogenetic methods were applied to human and other cultured mammalian cells to obtain a better understanding of the complex process of malignant cell transformation.

All direct acting carcinogens increased the frequency of SCE in Chinese hamster cells. Noncarcinogens were ineffective while indirect acting carcinogens increased SCE only in a cell-mediated system which permitted metabolic activation. It has been postulated that SCE are a result of post-replication DNA repair. Experiments with caffeine, a known inhibitor of post-replication repair, have shown that post-treatment with caffeine caused a significant enhancement in the frequency of chromosomal aberrations and SCE by "S" dependent agents such as alkylating agents or ultraviolet irradiation. If post-replication repair inhibition was the underlying mechanism for SCE, a reduction in SCE frequency would occur in the presence of caffeine.

One protocol for the treatment of psoriasis involves a clinical photochemotherapy regime designated PUVA, a combination of 8-mythoxysoralen (8-MOP) and long wavelength ultraviolet light (UVA). This combination is potentially deleterious to humans. The addition of the calculated therapeutic dose levels of 8-MOP and ultraviolet light will double the number of SCE's in a human lymphoblastoid cell line whereas neither chemical nor ultraviolet influences the SCE frequency. An important question now arises whether patients also develop a sensitivity to the photochemotherapy as indicated by an increase in SCE.

Lastly, the indepth cytogenetic analysis of Syrian hamster chromosomes constitution has been continued because of the relevance of Syrian hamster cells to neoplastic transformation. Additional information concerning heterochromatin characteristics of Syrian hamster cells was obtained by new methods for late replication by BrdU substitution and Giemsa stain. A new karyotype on the basis of heterochromatin was established. By use of denaturing agents such as sodium hydroxide or barium hydroxide, the autosomal centromeric heterochromatin was differentiated from the heterochromatin that composes the short arms of the autosomes. The genus Mesocrietus has, in addition to the Syrian hamster, two other species, M. newtoni and M. brandti. From karyotypic comparisons, it was concluded that the differences in chromosomes between species was due to Robertsonian fusion and translocations. Consideration of G and C-band analysis leads to the deduction that the golden hamster is probably the ancestrial species of this particular genus.

Significance to Biomedical Research and the Program of the Institute: The prevention of cancer in humans depends to a large extent on removing potentially harmful environmental agents. Accepted testing in animals is an insurmountable task. The proper evaluation of a chemical to determine if a chemical is carcinogenic requires a large number of animals and over two years, not including the time required to analyze the accumulated data. Bioassays capable of screening potential carcinogens in a relatively short period of time at a fraction of the current costs are essential to a workable testing program. The need to study chemical carcinogenesis *in vitro* is logical when one realizes that carcinogenesis is really a problem in cell ecology. Consequently, *in vitro* carcinogenesis provides an opportunity to study chemical-target cell interaction and chemical-nontarget cell problems such as other effects of the chemical on cells without the complications of *in vivo* problems. Therefore, *in vitro* carcinogenesis has wide applications in terms of studying

compounds in man's environment for the sake of control as well as for determining how they alter the physiological process of cells.

Proposed Course of Project: The conditions which are responsible for or can alter in vitro transformation with specific emphasis on the requirements of human fetal cells will be studied. Ultraviolet, X-rays, and combinations of carcinogens of different classes, the combination of in vivo - in vitro systems, will be used. The mechanisms of early events of carcinogen-cell interaction will be studied. Investigation of different proximate carcinogens and metabolic inhibitors is planned. The testing of the tumorigenicity of cells exposed for different time intervals will continue.

Publications

Adams, R. A., DiPaolo, J. A., and Homburger, F.: The Syrian hamster in toxicology and carcinogenesis research. Cancer Res. (In Press).

Casto, B. C., and DiPaolo, J. A.: Biological activity of polycyclic hydrocarbons for Syrian hamster cells in vitro. In Gelboin, H. V., and Ts'o, P.O.P., (Eds.): Polycyclic Hydrocarbons in Cancer: Chemistry, Molecular Biology and Environment, New York, Academic Press, (In Press).

Casto, B. C., Janosko, N., and DiPaolo, J. A.: Development of a focus assay model for transformation of hamster cells in vitro by chemical carcinogens. Cancer Res., 37: 3508-3515, 1977.

DiPaolo, J. A.: Chromosomal alterations in carcinogen transformed mammalian cells. In Ts'o, P. O. P. (Ed.): The Molecular Biology of the Mammalian Genetic Apparatus, North Holland, Amsterdam, Associated Scientific Publishers, Elsevier Excerpta Medica, Vol. II, 1977, pp. 205-227.

DiPaolo, J. A., and Casto, B. C.: Chemical carcinogenesis. In Gallo, R. C. (Ed.): Recent Advances in Cancer Research: Cell Biology, Molecular Biology, and Tumor Virology, Cleveland, CRC Press, Inc., Vol. I, 1977, pp. 17-47.

DiPaolo, J. A., and Casto, B. C.: In vitro carcinogenesis with cell strains in early passages. In Sanford, K., (Ed.): Third Decennial Review Conference on Cell Tissue and Organ Culture. Gene Expression and Regulation in Cultured Cells. Natl. Cancer Inst. Monogr., 48: 245-257, 1977.

DiPaolo, J. A., and Casto, B. C.: In vitro transformation of mammalian cells: Sequential treatment with diverse agents. In Slaga, T. J., Boutwell, R. K., and Sivak, A. (Eds.): Mechanisms of Tumor Promotion and Cocarcinogenesis, New York, Raven Press (In Press).

DiPaolo, J. A., and Donovan, P. J.: Transformation frequency of Syrian hamster cells and its modulation by UV-irradiation. In Hanna, M. G. (Ed.): International Conference on Ultraviolet Carcinogenesis. Natl. Cancer Inst. Monogr., (In Press).

DiPaolo, J. A., and Popescu, N. C.: Banding pattern analysis of initial structural chromosome alterations induced by N-methyl-N'-nitrosoguanidine in Syrian hamster cells. Mutat. Res., 44: 359-368, 1977.

DiPaolo, J. A., and Popescu, N. C.: Cytogenetics of Syrian hamster and its relationships to in vitro neoplastic transformation. In Homburger, F., (Ed.): The Syrian Hamster in Toxicology and Carcinogenesis Research. Progress in Experimental Tumor Research, Basel, New York, S. Karger (In Press).

Doniger, J.: The mechanism of post-replication repair in mammalian cells. In Hanawalt, P. C., Friedberg, E. C., and Fox, C. F. (Eds.): DNA Repair Mechanisms, (ICN-UCLA Symposia on Molecular and Cellular Biology, Vol. IX, New York, Academic Press, (In Press).

Hatch, G. G., Balwierz, P. J., Casto, B. C., and DiPaolo, J. A.: Characteristics of hamster cells transformed by the combined action of chemical and virus. Int. J. Cancer 21: 121-127, 1978.

Milo, G. E., Blakeslee, J., Yohn, D., and DiPaolo, J. A.: Biochemical activation of AHH activity, cellular distribution of polynuclear hydrocarbon metabolites, and DNA damage by PNH products in human cells in vitro. Cancer Res., 38: 1638-1644, 1978.

Myhr, B. C., and DiPaolo, J. A.: Comparison of mutagenesis and malignant transformation in vitro with methyl methanesulfonate and N-acetoxy-2-acetyl aminofluorene. Cancer Res., (In Press).

Myhr, B. C., and DiPaolo, J. A.: Mutagenesis by N-acetoxy-2-acetyl aminofluorene of Chinese hamster V79 cells is unaffected by caffeine. Chem.-Biol. Interact., (In Press).

Reitz, M. S., Saxinger, W. C., Ting, R. C., Gallo, R. C., and DiPaolo, J. A.: Lack of expression of type-C hamster virus after neoplastic transformation of hamster embryo fibroblasts by benzo(a)pyrene. Cancer Res., 37: 3583-3589, 1977.

Popescu, N. C., Turnbull, D., and DiPaolo, J. A.: Sister chromatid exchange and chromosome aberration using several carcinogens and noncarcinogens. J. Natl. Cancer Inst., 59: 289-293, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04673-07 BGY

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Neoplastic Transformation of Guinea Pig Cells by Chemical Carcinogens In Vitro.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. H. Evans	Head, Tumor Biology Section	BGY NCI
OTHER:	J. A. DiPaolo	Chief, Biology Branch	BGY NCI
	J. O. Rundell	Staff Fellow	BGY NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Biology Branch, Carcinogenesis Research Program

SECTION
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NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.6	1.6	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Carcinogens from diverse chemical classes induce neoplastic transformation of guinea pig fetal cells in culture. The transition to the neoplastic state often occurs in a stepwise manner. Morphological transformation frequently precedes neoplastic transformation, the ability of the cells to produce progressively growing tumors in syngeneic guinea pigs, by several months or more. Neoplastic transformation, furthermore, occurs four or more months after carcinogen treatment. Alterations in plating efficiency, growth rate, and chromosome number and structure are not necessary for the development of neoplastic transformation. The ability of transformed cells to grow as colonies in agar, susceptibility of the cells to cytotoxic effects of lymphotoxin, intradermal skin reactivity of transformed cells in nonimmune guinea pigs, and secretion of large amounts of plasminogen activator are characteristics that correlate well with neoplastic transformation. These cell properties are useful indicators of the tumorigenic potential of guinea pig cells transformed by chemical carcinogens and for study of somatic cell alterations essential for the development of the neoplastic state.

PHS-6040
(Rev. 10-76)

Project No. Z01 CP 04673-07 BGY

Project Description

Objectives: The primary objective of this project is to establish conditions and methods for the quantitative study of *in vitro* chemical transformation of guinea pig cells. The investigation complements the previously developed and current *in vitro* chemical carcinogenesis system established within the Biology Branch employing Syrian hamster embryo cells and the tumor immunology program utilizing *in vivo* chemical carcinogen induced tumors developed in strain-2 guinea pigs.

The specific objectives of the project are two-fold:

1. The development of a rapid assay system for chemical carcinogens suitable for the screening of compounds and populations. As an inbred strain with a low incidence of spontaneous malignancy, strain-2 guinea pigs offer a mammalian system in addition to the Syrian hamster for the analysis of chemicals as potential carcinogens and investigation of the mechanisms of chemical carcinogenesis at the cellular level.

2. The development of assays to determine whether *in vitro* chemical transformation is accompanied by the appearance of tumor specific antigens and/or other alterations associated with the neoplastic state. The Biology Branch has extensive experience with tumor immunology in strain-2 guinea pigs. A variety of *in vivo* and *in vitro* immunological techniques are presently being utilized. The availability of these techniques and the syngeneic system offered by the inbred strain-2 guinea pig make this system a better choice for immunological study at this time than the Syrian hamster. The development of neoplastic cells transformed *in vitro* by chemical carcinogens will permit assessment of cell surface antigen alterations associated with chemical carcinogens as well as further understanding of *in vivo* tumor immunity in strain-2 guinea pigs as a model system for carcinogenesis and tumor immunology in general.

Methods Employed: The quantitative *in vitro* chemical carcinogenesis system developed employing Syrian hamster embryo cells within the Biology Branch has been utilized throughout this study. Cells are freshly isolated from whole or specific organs of strain-2 guinea pig embryos or fetuses and are grown in the presence or absence of irradiated rat, hamster or strain-2 guinea pig feeder cell layers. The cells as a mass culture or as individual cells are exposed to chemical carcinogen subsequent to seeding the cells in the Petri dish.

Major Findings: 1. Carcinogens from diverse chemical classes including the polycyclic aromatic hydrocarbons benzo(a)pyrene, 3-methylcholanthrene, and 7,12-dimethylbenz(a)anthracene, the amide N-acetoxy-N-2-fluorenylacetamide, the nitroso compound N-methyl-N'-nitro-N-nitrosoguanidine, the nitrosamine diethylnitrosamine and aflatoxin B_1 induce neoplastic transformation of strain-2/N fibroblast-like guinea pig cells in culture. Carcinogenesis can be iniated by application of the carcinogen directly to cells in culture except for compounds such as diethylnitrosamine that require metabolic activation.

These compounds and the direct acting carcinogens are effective when the cells are exposed in vivo with the host mediated in vivo - in vitro method.

2. Carcinogenesis utilizing guinea pig cells proceeds in discrete stages that frequently are displayed for extended periods of time. Morphological alterations relative to controls develop shortly after carcinogen exposure and persist for months. After four or more months, morphologically altered cells proceed to the stage of morphological transformation characterized by random growth and piling up of cells. Neoplastic transformation, the ability of cells to produce tumors when inoculated into host animals, may be demonstrated at the time of morphological transformation or frequently may require additional months of subculture. This stepwise progression of the carcinogenic process occurs following treatment of cells obtained from fetuses in midterm and near-term gestation and with cells isolated from neonatal guinea pigs. A similar pattern was found during in vitro carcinogenesis of a non-tumorigenic morphologically oriented fibroblast-like guinea pig continuous cell line treated with carcinogen 14 months after introduction into culture. This indicates that the transformation frequency, latent period, and multiple stages characteristic of carcinogenesis can be independent of cell age or number of generations.

3. A number of somatic cell alterations including the ability to form colonies in agar, secretion of plasminogen activator, intradermal skin reactivity in nonimmune syngeneic animals and susceptibility to the cytotoxic activity of lymphotoxin develop in close proximity or concomitantly with neoplastic transformation. These cell properties provide useful quantitative means to identify neoplastic transformation of cells in culture and to investigate cellular characteristics essential to the development of the neoplastic state.

4. The neoplastic state is associated with a quantitatively specific susceptibility to lymphotoxin. There is a wide range of lymphotoxin susceptibility among different neoplastically transformed cells that is independent of the origin of the cells or the initiating chemical carcinogen. Neither chromosomal alterations nor detectable tumor specific cell surface neoantigens are necessary for neoplastic transformation; furthermore, these factors are unrelated to lymphotoxin susceptibility. Investigations during the past year have shown that colony formation in agar and lymphotoxin susceptibility develop concomitantly with or following the onset of the stage of preneoplastic morphological transformation. They can develop independently of one another and many cell generations and months prior to attaining the stage of neoplastic transformation. Natural intradermal skin reactivity, however, has yet to be observed until the stage of neoplastic transformation. This separation of lymphotoxin susceptibility and natural skin reactivity into two different stages of carcinogenesis is of interest as these are two potential points for immunobiologic modulation of in vivo carcinogenesis.

Significance to Biomedical Research and the Program of the Institute: This project provides an unique in vitro model system with several distinguishing characteristics for the study of chemical carcinogenesis. In vitro chemical neoplastic transformation of strain-2/N guinea pig cells will provide another assay system in addition to the Syrian hamster and established mammalian cell lines for screening of chemicals as potential carcinogens and broaden our

understanding of carcinogenesis at the cellular level through inspection of intracellular and cell surface alterations accompanying neoplastic transformation. Development of this new model offers an additional avenue for studying the process of carcinogenesis using a species with established tumor biology in which spontaneous transformation has not been seen, which possesses well defined immunological parameters, and in which discrete stages in the transition from the growth controlled to the neoplastic state may be studied.

Proposed Course of Project: Conditions and methods will continue to be refined toward attaining optimal development of *in vitro* transformation of strain-2 guinea pig cells as a reproducible, quantitative and reliable assay for potential chemical carcinogens. Investigations will continue in defining the events, factors and cellular alterations accompanying the stages, i.e. morphologic alteration, morphologic transformation, and potential for neoplastic growth, in the transition from the growth controlled to the neoplastic state. Of immediate interest are the properties of cells with neoplastic potential that permit them to grow as colonies in agar and to be susceptible to the cytotoxic and growth inhibitory activities mediuated by nonimmune leukocytes in the absence of or independent of the presence of detectable tumor specific cell surface antigens. The elucidation of these relationships will provide further understanding of the mechanism of chemical carcinogenesis at the cellular levels of tumor establishment and immunity in the host animal.

Publications

Evans, C. H.: Neoplastic transformation by chemicals of guinea pig fetal cells in culture. In Saffiotti, U., and Autrup, H. (Eds.): *In Vitro Carcinogenesis. Guide to the Literature, Recent Advances and Laboratory Procedures.* National Cancer Institute Carcinogenesis Technical Report Series No. 44 (1978), DHEW Publication No (NIH) 78-844, U. S. Gov't. Printing Office, Washington, D. C., pp. 82-83.

SUMMARY REPORT

CARCINOGEN METABOLISM AND TOXICOLOGY BRANCH

October 1, 1977 through September 30, 1978

"Plans, develops, and conducts a research program including (1) identification, chemical analysis, mode of formation, and metabolism of various classes of chemical carcinogens; (2) studies on the toxicology and metabolic pathways of carcinogens in selected animal systems and in relation to man; (3) correlation of chemical and toxicological data for the selection and screening of chemical carcinogens; and (4) development of biological and biochemical methods for the identification of reactive carcinogenic metabolites."

The Carcinogen Metabolism and Toxicology Branch (CMT) is involved both in a number of inhouse research projects and in the scientific direction of collaborative projects through different areas of the Carcinogenesis Research Program.

The collaborative projects are conducted through contracts on the toxicology and metabolism of carcinogens (aromatic amines, some halogenated aliphatic compounds, aflatoxin and derivatives), and part of the program on nitrosamines. Branch staff are involved in several projects on nitrosamines and in the direction of the repository of chemical carcinogens. Branch staff are also involved in the selection of compounds for the Carcinogenesis Testing Program and in the review for this program. In addition staff of the Branch act as monitors for one of the CREG programs and serve as project monitors and coordinators for the program at the FCRC. Furthermore, Branch members are consultants for various national and international activities in the area of chemical carcinogenesis and environmental carcinogens, and act as government witnesses for hearings on various environmental chemicals.

The research activities in the Office of the Chief are directed largely toward studies on metabolism and activation of various classes of chemical carcinogens. Studies on the role of certain nutritional factors in the mechanism of action of carcinogens are also being investigated. Some small scale bioassays on structure-activity relationships are being conducted. In addition, the physical-chemical differences between normal and transformed cells with respect to certain physical parameters are being investigated.

An investigation on the metabolism of the environmental material 2,4-toluenediamine, an intermediate for the polyurethan foam, has been written up for publication in a journal. As further reference materials and other possible metabolites become available this project may be reopened in order to complete some aspects of the problem.

It was definitely found that N-formylation of 2-aminoanthraquinone occurred in rats fed this dye intermediate, but the mechanism is unknown. An attempt was made to extend this finding during metabolic study of 2,4-diaminoanisole. However, it was found that the presumed N-formyl derivative of 2,4-diaminoanisole was only an artifact resulting from the solvent used in the extraction procedure.

The study of 2,4-diaminoanisole, a mutagenic compound used in hair dye preparations, has continued. The compound is excreted very rapidly; acetylation of the amino groups and, to a minor extent, demethylation had occurred.

Differences in the metabolism of the compound in rats fed a normal diet and those fed a diet high in the antioxidant BHT (butylated hydroxytoluene) are being followed.

The effects of L-tryptophan and various of its metabolites on the enzyme azo reductase which is important in the detoxication of azo dyes have been followed. The three metabolites which did inhibit the enzyme were found to do so by virtue of their autooxidation to the phenoxazine compounds. Further investigations in this aspect of the problem are continuing.

Analytical Chemistry Section - *"Conducts independent research on the organic, analytical, and biological chemistry of nitrosamines and related compounds and collaborates with other members of the Carcinogenesis staff in matters requiring chemical expertise, especially in problems of identifying non-polymeric organic molecules."* Activities for the past year have focused on two main areas:

N-Nitroso Compounds. (a) A variety of metal nitrites and nitrosyls have been shown to nitrosate amines under conditions simulating those found in cutting fluids and other nitrosamine-contaminated media in the human environment. (b) Evidence has been found suggesting that even ammonia must now be considered a potential nitrosating agent. (c) Fully deuterated dimethylnitrosamine is metabolized as rapidly as the unlabeled derivative in vivo; however, given a choice between the labeled and unlabeled compounds (administered in large doses as the equimolar mixture), rats metabolize the unlabeled material four times as fast. Apparently, the product determining step in metabolism under these conditions (but not the rate-determining step) involves cleavage of carbon-hydrogen (deuterium) bond. (d) The methylating agent produced during microsomal attack on the dimethylnitrosamine molecule in vitro either exchanges its hydrogen isotope completely with the medium at some point in its formation (in contrast to in vivo metabolism), or else the methyl groups actually transferred originate exclusively from the medium, rather than from the nitrosamine. Formaldehyde detected in this reaction also comes largely from the medium.

Mass Spectrometry. The mass spectrometry laboratory is involved in carrying out independent research as well as participation in collaborative projects with a number of investigators on carcinogenesis related problems, where mass spectrometry, as an analytical tool, can be applied. (a) High resolution studies indicate that α-acetoxylated dialkylnitrosamines undergo molecular rearrangements in the gas phase to diazonium ions, protonated diazoalkanes,

alkane diazotic acid and to ions corresponding in composition to the acetate of alkyldiazotic acid. The formation of these species may have relevance to the solution chemistry of α-oxygenated dialkylnitrosamines. (b) Using deuterium labeled substrates and reagents the detailed mechanism of enzymatic formation of the three dihydrodiols and of the 3-, 7-, and 9-phenols of benzo[a]pyrene has been elucidated. Evidence is put forward for enzymatic formation of the transient 2,3-epoxide intermediate, in collaborative work with the Chemistry Branch of the Division. Also in a collaborative effort mass spectra on metabolites from labeled and unlabeled benz[a]anthracene allowed direct identification of the 5,6-epoxide, of the anti 8,9-diol-10,11-epoxide among others, and of the inferred existence of the unstable 3,4-epoxide of benz[a]anthracene. (c) In supporting the projects within the Branch, mass spectral measurements on a number of synthetic intermediates and on metabolites from 2,4-diaminotoluene and 2,4-diaminoanisole have facilitated chemical structure determinations. (d) In a collaborative effort with the Fermentation Laboratory, FCRC, on structural studies of potential chemotherapeutic agents, detailed mass spectral analysis has been completed on several griseorhodin analogs and on some ansamycin type antibiotics. (e) Efforts are being continued in the analysis of bioactive materials that may play a role in cancer cause and prevention mechanisms, such as vitamin A derivatives and substances with immunological properties, e.g. multiacylated trehalose derivatives.

Nutrition and Metabolism Section - *"Plans, develops and conducts research on (1) the effects of dietary constituents on the activities of various classes of chemical reactivities of ultimate carcinogens; (2) the metabolism of dietary components known to modify the carcinogenic process; and (3) the correlation of chemical and toxicological data for the identification of chemical carcinogens."* The section has focused its interest on the role of the lipotropes methionine, choline, vitamin B_{12} and folic acid in chemical carcinogenesis. The growth of transformed rat liver epithelial cells has been shown to be markedly inhibited by the replacement of methionine by homocysteine in the medium; normal liver cells are much less affected by the change. The elevated methionine requirement by transformed cells could not be generally ascribed to a secondary folate deficiency or to a deficiency of polyamines. However, one transformed line did not survive at methionine levels which supported the growth of normal hepatocytes; a second transformed line had exceedingly low levels of methionine synthetase. Thus, the elevated methionine requirement of transformed cells appears to result from more than one intracellular biochemical abnormality. Similar to skin epithelial cells in vivo, liver epithelial cells in culture treated with phorbol esters, show an increased incorporation of choline and ethanolamine into the corresponding phospholipids.

The section's research activities have also included an examination of species, sex and strain sensitivities to the formation and inhibition of tumors, and investigations of structure-activity relations in chemical mutagenesis and carcinogenesis. The mutagenic activity in the Ames assay of a series of substituted organo halides was compared to their tumorigenic activity in the mouse lung adenoma test. Nucleophilic substitution within alkyl chlorides tended to increase both their mutagenic and carcinogenic

activities. Further, the tumorigenic activity of the organo halides tended to be proportional to their mutagenic activity and to the dose that could be administered. A review of NCI's initial studies on mutagenesis and carcinogenesis indicated that a combined prescreening system including Ames' *Salmonella* strains and polymerase A-deficient *E. coli* detects 81% of the carcinogens tested.

Studies to compare the toxicity, metabolism and carcinogenic activity of tyrosine and of ethionine in mice and rats have begun. Tyrosine appears to induce hyperplasia in the livers of rats. Since methionine has been shown to inhibit the toxic effects of both ethionine and tyrosine, rats were fed an amino acid defined diet in which the content of methionine and its precursors (choline, vitamin B_{12} and folic acid) may be controlled. Preliminary results show the expected synergy with multiple deficiencies of methyl donors.

The role of sex hormones as potential promoters is being investigated. Testosterone was shown to increase and estradiol to inhibit the induction of ornithine decarboxylase in the livers of rats treated with growth hormone.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04589-01-CMT

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Endocrine Factors in Tumor Promotion

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Co-PI:	Dr. Lionel A. Poirier	Head, Nutr. & Met. Sec.	CMT	NCI
OTHER:	Dr. Richard S. Yamamoto	Chemist	CMT	NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Carcinogen Metabolism and Toxicology Branch, Carcinogenesis Research Program

SECTION
Nutrition and Metabolism Section

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of hormones on the induction of ornithine decarboxylase (ODC) in vivo and in liver epithelial cells in culture are being studied. Previous studies have shown that ODC induction may be a marker for tumor promoters. Hormones often increase the activities of complete carcinogens. ODC is used to study the degree of biochemical similarity between hormones and promoters. The present studies indicate that growth hormone and prolactin each induce high levels of ODC in the liver and kidneys of both male and female rats, while cortisone induces hepatic ODC only. Estradiol inhibits, while testosterone increases, the ODC induction by growth hormone. Hormonal effects on tissue levels of S-adenosylmethionine decarboxylase and polyamines will also be determined.

PHS-6040
(Rev. 10-76)

Project No. Z01-04589-01-CMT

Project Description

Objectives: The major objectives of these studies is to determine whether the enhancement of the activity of certain complete carcinogens by hormones (e.g. testosterone in hepatocarcinogenesis) is due to a promoting activity of the hormone.

Previous studies have demonstrated that certain hormones often increase the activity of specific carcinogens towards their target organs. In theory, such effects may be due to (a) altered metabolism, increasing the effective intracellular concentration of ultimate carcinogens, (b) promotion of initiated cells and (c) other incompletely defined co-carcinogenic effects. Other studies have shown that hormones frequently alter carcinogen metabolism. We are attempting to show that hormones may act as promoters. The induction of ornithine decarboxylase (ODC) is used as marker of promoting-like activity.

Methods Employed: The enzymes ODC and S-adenosylmethionine decarboxylase are assayed by trapping labelled CO_2. Tissue levels of polyamines are determined by thin layer chromatography and fluorimatic analysis. The enzymes and the polyamines are determined in hepatocytes in vitro and in multiple organs of intact and hormonally-oblated rats, mice and hamsters, which have been treated with hormones.

Major Findings: (a) Testosterone enhances and estradiol inhibits the induction of ODC by growth hormone in the livers of rats. This finding is consistent with a promoting effect of these 3 hormones in hepatocarcinogenesis. (b) Sex differences can not be observed in the ODC and S-adenosylmethionine decarboxylase levels in 13 organs of intact rats.

Significance to Biomedical Research and the Program of the Institute: A major goal of the NCI is to determine the mechanism of action of chemical carcinogens. These studies attempt to clarify the contribution of hormones to the carcinogenic process in man.

Proposed Course of the Project: To test the hypothesis that hormones often act as promoters, the following investigations will be pursued: 1) Studies of ODC induction by hormones will be extended and compared to the effects of the same hormones on DNA-synthesis and cell mitosis in the same organs. 2) The predictability of hormonal induction of ODC as a guide to tumor promoting activity by hormones will be tested by initiation-promotion experiments. Ultimate carcinogens will be used as initiators and hormones as promoters, in those tissues in which they induce ODC.

Project No. Z01-04589-01-CMT

Publications

Nawata, H., Kato, K. I. and Ibayashi, H.: Age-dependent change of serum 5α-dihydrotestosterone and its relation to testosterone in man. Endocrinol. Japan. 24: 41-45, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04493-01-CMT

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Physicochemical Studies of Normal and Transformed Epithelial Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. E. Kaplan	Biochemist	CMT NCI
OTHER:	M. Yamaguchi	Visiting Fellow	CMT NCI
	M. Wilson	Chemist	CMT NCI
	E. Weiss	Student Intern	CMT NCI
	P. A. Margolis	Student Intern	CMT NCI

COOPERATING UNITS (if any)
Dr. Richard B. Setlow, ERDA Brookhaven National Lab., Upton, N.Y.; Dr. Martin Lipkin, Memorial Hospital, New York, N. Y.; Dr. George W. Ellison, Dept. of Neurology, Univ. Calif., Los Angeles; Dr. Neblon Freeman, Walter Reed Hospital; Charles Hanna, NIAMDD, NIH, G. Knott, DCRT, NIH; R. Feldman, DCRT, NIH

LAB/BRANCH
Carcinogen Metabolism and Toxicology Branch, Carcinogenesis Research Program

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 2.5	PROFESSIONAL: 2.0	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Physico-chemical studies of differences between normal and tumor-producing cells include: 1) The evaluation of morphological differences between normal and transformed hepatocytes by electron microscopy. 2) The study of changes in the kinetic behavior of lactate dehydrogenase isoenzymes (LDH) from these cells as a source of the increased excretion of acid observed upon transformation. 3) The comparison of kinetic and electrophoretic behavior of LDH isoenzymes in normal and transformed hepatocytes. 4) The analysis of this enzyme from mammalian tissue sources for comparison of electrophoretic and kinetic behavior with cells grown in culture and for clinical application of the methodology. 5) The methods were developed using LDH-1, -2 and -3 isolated from grey matter from exsanguinated rabbit brain. These results indicate that the kinetic behavior of isoenzymes from different tissues is unique and cannot be predicted on the basis of parallel migration in an electrical field. 6) The study of the effect of cyclocytidine upon DNA repair in normal and xeroderma pigmentosum human fibroblasts (in collaboration with R. Setlow).

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04493-01-CMT

Project Description

Objectives: Rat hepatocytes, grown in culture free of fibroblasts, have transformed spontaneously or as a result of exposure to chemical carcinogens to produce tumors when injected into Fischer rats. These transformed lines differ from normal cells and from each other when examined by light microscopy, but all are characterized by the increased production of acid during growth. Further details of the morphological changes occurring with transformation are under examination by electron microscopy, with special emphasis being placed on preserving the intercellular structures for observation. The abnormal production of acid may be related to changes in the kinetic behavior of lactate dehydrogenase isoenzymes during transformation. Measurements underway indicate that such changes do take place. The kinetic behavior of LDH isoenzymes and the electrophoretic separation of LDH isoenzymes from normal and transformed hepatocytes will be evaluated. This information will be compared with isoenzymes from mammalian tissue sources to see if changes occur due to growth in culture and also for possible clinical application of these findings. Measurements with rabbit brain isoenzymes already indicate that kinetic measurements are more sensitive to molecular differences than electrophoretic behavior as a means of identifying enzymes from different tissues.

Previous studies (A. E. Kaplan and R. A. Sanchez, The Salk Institute) revealed that cyclocytidine, a cyclized derivative of cytosine arabinoside, inhibited DNA synthesis under conditions normally leading to the formation of lymphocytes as a response to delayed hypersensitivity. In order to identify the steps in the synthetic pathway affected by this compound, collaborative studies of cellular DNA damage and repair systems were begun with Dr. Richard B. Setlow. The investigation at NCI will be concerned with analyses to evaluate binding reactions of the compound with DNA and with building three-dimensional models for evaluation of the physical results. These findings will be used in conjunction with the cell studies in Dr. Setlow's laboratory as a means of obtaining further information regarding the action of this nucleoside.

Methods Employed: A number of physico-chemical methods are used in these studies of normal and transformed hepatocytes. (1) These cells grow in monolayers and special effort is being made to retain intercellular structure in fixing and embedding these preparations for electron micrography to provide the maximum information regarding morphological differences among the transformed cell lines. (2) The Aminco-Morrow Stopped Flow DASAR System is used in the LDH analyses for the collection of kinetic data with cellular extracts and purified isoenzymes. These data are then analyzed by computer with the MLAB on-line modelling system developed by G. Knott et al. DCRT, NIH. (3) The kinetic results will be compared with electrophoretic separations which serve for the numerical identification of the LDH isoenzymes. These methods have been developed in studies with LDH isoenzymes from rabbit brain.

Studies with cyclocytidine to be carried out in this laboratory include NMR analysis for detection of binding reactions and computerized three-dimensional modelling of the nucleoside by the methods developed by R. Feldman, DCRT, NIH.

Major Findings: Kinetic analysis shows the LDH isoenzymes in cytosol preparations from normal and transformed cell hepatocytes differ. This has been found in one cell line which is a spontaneous transformant and in a second cell line resulting from exposure of normal cells in vitro to nitrosomethylurea. In all three extracts examined thus far complex kinetics are seen in the MLAB analyses, as would be expected with a family of iso-enzymes. The rate of decay of the reaction of the isoenzymes from chemically transformed cells is twice that of the preparation from normal cells.

LDH-1, -2 and -3 from brain differ markedly in kinetic behavior from LDH-1 and -2 from cardiac tissue, but are much more similar to LDH-5 from liver. These results indicate that structure at the catalytic site may be a far more unique development in different organs than overall charge which determines electrophoretic migration.

Studies with cyclocytidine indicate that the UV-induced endonuclease reactions involved in excision repair are inhibited by this nucleoside, leading to the isolation of fragmented DNA.

Significance to Biomedical Research and the Program of the Institute: Physico-chemical analyses of LDH, a family of isoenzymes normally found in liver and other tissues, indicate that a fundamental change in the catalytic behavior of these molecules takes place with transformation. From previous results comparing enzymes of similar electrophoretic mobility (LDH-1 and -2) from heart and brain, it has been shown that kinetic analysis can identify molecular differences even where overall charge distribution results in equal rates of migration in electrical fields. Thus the implications of the kinetic differences observed in transformed versus normal hepatocytes point to fundamental structural alterations at the catalytic site of the enzyme(s) which can be measured and which can serve as a means of detecting early stages of transformation in vivo without depending upon specific morphological changes in the cell for identification. Such new kinds of analyses will serve to increase the range of evaluations for early diagnosis of cancer.

Studies with cyclotidine in vitro indicate that the nucleoside interferes with enzyme reactions which repair DNA. These results support previous observations in vivo and have important chemotherapeutic implications for the use of this compound.

Proposed Course of Project: Examination of available hepatocyte lines for identification of morphological differences between normal and transformed lines by electron microscopy will be carried out. The same cells will be

studied for kinetic and electrophoretic differences in the LDH isoenzymes. The enzyme methods will be applied to isoenzymes from tissue sources for development of these methods for clinical evaluations, especially with respect to metastases to the central nervous system.

The studies with cyclocytidine will be extended to characterize the molecular basis for the action of the compound in inhibiting DNA synthesis in vivo and in vitro.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04510-05-CMT

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Environmental and Genetic Factors in Digestive System Carcinogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Richard S. Yamamoto	Research Chemist	CMT	NCI
OTHER:	Hajime Nawata	Visiting Associate	CMT	NCI
	Susan Rector	Chemist	CMT	NCI

COOPERATING UNITS (if any) Dr. Martin Wenk, Microbiological Associates, Bethesda, Md.

LAB/BRANCH
Carcinogen Metabolism & Toxicology Branch, Carcinogenesis Research Program

SECTION
Nutrition & Metabolism Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	1.0	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A study of strain and sex susceptibility to colon carcinogenesis using 1,2-dimethylhydrazine (DMH) and azoxymethane (AOM) in mice and rats. Female rats were resistant to DMH carcinogenesis but not AOM, developing both colon and kidney tumors with AOM. Piperazine, a pinworm eradicator, altered the incidence of colon tumors produced by DMH and AOM in rats.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04510-05-CMT

Project Description

Objectives: To determine interrelationships of environmental and genetic factors in the development of gastrointestinal tract carcinogenesis. To study the role of hormones and dietary factors in digestive tract carcinogenesis.

Methods Employed: Various strains of rats and mice are compared for their susceptibility to DMH and AOM carcinogenesis. Tumor development and other physiological changes are observed. The affect of dietary factors on colon carcinogenesis is also tested.

Major Findings: (1) Response of different strains of mice on AOM and DMH. BALB/c strain of mice was tested for its response to AOM and DMH. Colonic tumors developed rapidly at a very low dose, 0.1 mmole, of DMH and AOM. Most of the tumors were polypoid, resembling polyposis in man.

(2) Effects of piperazine on colon carcinogenesis. Piperazine, given in drinking water, did not affect AOM and DMH colon tumorigenesis, but did decrease the incidence of tumors. The presence of pinworms did not affect colon tumorigenesis.

(3) Response of hamsters to pancreatic duct carcinogenesis. The Syrian golden hamsters receiving a very low dose of 2,2'-dihydroxypropane-nitrosamine for only 20 weeks developed pancreatic ductal carcinomas more rapidly on a semi-purified diet than the regular laboratory chow.

Significance to Biomedical Research and the Program of the Institute: The high morbidity and mortality from gastrointestinal cancer is a major health problem in the United States. The development of animal models of gastrointestinal neoplasms, and their use for the study of etiological factors which act in combination of factors which modify the incidences and development of such tumors, is expected to lead to knowledge relevant to their prevention.

Proposed Course of Project: Further studies of dietary ingredients, antioxidants, antibiotics and enzyme inhibitors will be made in conjunction with compounds known to produce gastrointestinal tract cancer. Resistant vs. susceptible strains and species will be used to study the metabolic activation of the colon carcinogens. The influence of dietary components on pancreatic carcinogenesis by DIPN will be concluded. Further studies of the role of hormones in chemical carcinogenesis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04542-06-CMT

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Chemistry of N-Nitroso Compounds

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. Keefer	Head, Anal. Chem. Sec.	CMT NCI
OTHER:	P. Roller	Chemist	CMT NCI
	R. Angeles	Staff Fellow	CMT NCI
	A. Croisy	Visiting Associate	CMT NCI
	S. Uhm	Visiting Fellow	CMT NCI
	J. Miller	Chemist	CMT NCI
	C. Michejda	Head, Chem. of Carc.	FCRC
	M. Kroeger-Koepke	Chemist	FCRC
	D. Wilbur	Chemist	FCRC
	P. Swann	Courtauld Inst., London	

COOPERATING UNITS (if any)

None

LAB/BRANCH
Carcinogen Metabolism and Toxicology Branch, Carcinogenesis Research Program

SECTION
Analytical Chemistry Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 3.5	PROFESSIONAL: 3.0	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Data concerning the chemical and physical properties of the carcinogenic N-nitroso compounds will be collected. Specific emphases will include studies of: a) catalysis of N-nitrosation reactions of environmental interest by metal species; b) mechanisms of microsomal attack on the dialkylnitrosamines, with comparison of results to in vivo metabolic data; c) preparation of novel nitrosamines and their derivatives for chemical and biological studies. Possible implications of this work with respect to the overall goal of human cancer prevention will be considered.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04542-06-CMT

Project Description

Objectives: (1) To investigate mechanisms by which metal ions might promote the formation of N-nitroso compounds in the environment. (2) To study mechanisms of metabolism and carcinogenesis by N-nitroso compounds using deuterium labeled substrates. (3) To synthesize models of possible metabolites of certain N-nitroso compounds for comparisons of their physical, chemical and biological properties with those of the parent procarcinogens. (4) To study mechanisms of possible artifact formation during analysis for environmental N-nitroso compounds.

Methods Employed: The standard methods of synthetic and mechanistic chemistry have been used in these investigations.

Major Findings: Several transition metal complexes have been shown to promote nitrosamine-forming reactions between nitrite ion and secondary amines at alkaline pH; the results are proposed as models for studying mechanisms of nitrosamine contamination in commercial cutting fluids and human saliva. Metal nitrites and nitrosyls which nitrosate amines in non-hydroxylic solvents have been proposed as models of critical intermediates in the production of dimethylnitrosamine and nitrosopyrrolidine in frying bacon. Ruthenium (III) pentammine nitrosyl ions can be used to nitrosate amines in strongly alkaline medium; since the starting metal complex can be prepared by autoxidation of the corresponding hexammine under these conditions, even the -3 (ammonia) oxidation state of nitrogen must be regarded as having nitrosating potential.

In vitro metabolism of Z-methyl(methyl-d_3)nitrosamine [DMN-d_3] by rat liver microsomes proceeds at a rate roughly equal to that of dimethylnitrosamine [DMN], rather than that of dimethylnitrosamine-d_6 [DMN-d_6] at all concentrations studied; thus each enzyme responsible for significant microsomal metabolism of DMN stereoselectively attacks the methyl group anti to the nitroso nitrogen atom. Attempts to confirm this by trapping the alkylating agent presumably produced during this reaction as 3,4-dichlorothioanisole led to the interesting finding that none of the rather abundant alkylation product isolated could be shown to contain deuterium, even when DMN-d_6 was used as a substrate; this suggests that either (1) all the methylating agent trapped comes from the medium, or (2) in contrast to the corresponding in vivo reaction, the methylating agent undergoes hydrogen isotope exchange with the medium at some point before dichlorothioanisole is formed. Variable proportions (but not all) of the formaldehyde formed in these reactions comes from the medium as well.

In vivo, DMN-d_6 is not a competitive inhibitor of DMN, as measured by administering the carcinogen to rats at a 40 mg/kg dose level and following carbon dioxide production. However, a primary isotope effect of k_H/k_D = 4 on the rate of CO_2 production was observed when mixtures were administered to the animals; this value is comparable to the initial isotope effect on base alkylation of nucleic acids in vivo.

In contrast to recent literature reports, nitric oxide is an excellent nitrosating agent for certain amines. The Z⇌E interconversion of dialkylnitrosamines has been shown to be acid catalyzed. DMN can be anodically oxidized to dimethylnitramine. At least part of the DMN formed when nitrite is passed through a mixed-bed ion exchange resin appears to be the result of a simple, acid-catalyzed nitrosation.

Significance to Biomedical Research and the Program of the Institute: With considerable public attention focused on the possibility that N-nitroso compounds might be responsible for some human cancer, collection of data on the chemical, physical, and biological properties of this important class of compounds is an urgent matter. Certain aspects of the studies reported here may aid in developing methods for predicting, analyzing, and preventing environmental contamination by these potential carcinogens, while other aspects are aimed at shedding light on the biochemical mechanisms by which they exhibit their untoward effects.

Proposed Course of Project: The studies described above will be completed and published, and the important hypotheses and implications they suggest will be investigated as appropriate.

Publications

Gaffield, W., Keefer, L. K. and Roller, P. P.: Synthesis of the selective bladder carcinogen, N-(n-butyl)-N-(3-carboxypropyl)nitrosamine (BCPN). Organic Preparations & Procedures Int. 9: 49-52, 1977.

Gaffield, W., Roller, P. P., Palmer, W. G. and Keefer, L. K.: Tritium gas exposure as an alternative to base-catalyzed exchange for the one-step tritiation of nitrosamines. J. Labeled Comp. & Radiopharmaceut. 14: 91-97, 1978.

Reese, D. H., Friedman, R. D., Gaffield, W. and Keefer, L. K.: Focal suppression and induction of hyperplasia by the bladder carcinogens butyl(4-hydroxybutyl)nitrosamine and butyl(3-carboxypropyl)nitrosamine in organ-cultured rat bladder epithelium. J. Nat. Cancer Inst. 60: 219-223, 1978.

Angeles, R. M., Keefer, L. K., Roller, P. P. and Uhm, S. J.: Chemical models for possible artifactual nitrosamine formation in environmental analysis. IARC Scientific Publications (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04580-04-CMT

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

The Role of Lipotropes in Carcinogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Lionel A. Poirier	Head, Nutrition & Metabolism Section	CMT	NCI
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	H. J. Raj	Visiting Fellow	CMT	NCI
	Camille Hyde	Staff Fellow	CMT	NCI
	Richard Yamamoto	Chemist	CMT	NCI
	Susan Rector	Chemist	CMT	NCI
	Lawrence Lanier	Biological Laboratory Technician	CMT	NCI

COOPERATING UNITS (if any) Dr. J. C. Linnell, Westminster Square Hospital, London
Dr. Eli Seifter, Albert Einstein College of Medicine, New York

LAB/BRANCH
Carcinogen Metabolism and Toxicology Branch, Carcinogenesis Research Program

SECTION
Nutrition & Metabolism Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	1.7	2.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The mechanisms behind the alteration of chemical carcinogenesis by the dietary lipotropes, choline, methionine, folic acid and vitamin B_{12} have been studied. The chronic administration of diethylnitrosamine (DEN) to rats led to an increased uptake of labelled precursors into hepatic lecithin, while the uptake of labelled precursors into phosphatidyl ethanolamine and phosphatidyl serine was unaffected. The effects of promoters on phospholipid biosynthesis are being investigated. The elevated methionine requirement of transformed liver epithelial cells appears to be the consequence of more than one biochemical abnormality. The metabolism and carcinogenic activity of ethionine in different species is being compared.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04580-04-CMT

Project Description

Objectives: Folic acid and the dietary lipotropes methionine, choline, and vitamin B_{12} significantly modify the production of tumors by certain chemical carcinogens. The extent of and the mechanisms behind these effects are being investigated. The effects of the lipotropes on chemical carcinogenesis by a variety of agents are under investigation. In addition, the mutual metabolic interactions of the lipotropes and the chemical carcinogens are to be determined.

Methods Employed: The carcinogenic activities of several standard carcinogens were compared in rats fed defined diets. S-Adenosylmethionine and S-adenosylethionine are determined using isotope-dilution techniques and thin layer chromatography followed by microbiological assay. Phospholipid metabolism was measured by standard extraction, colorimetric and chromatographic techniques. The nutritional requirements of normal and transformed liver epithelial cells were determined in tissue culture systems developed in this laboratory.

Major Findings: The chronic administration of trifluoromethionine, a methionine antagonist, did not produce tumors in rats. Phorbol esters which are tumor promoters stimulated the uptake of choline into the phosphatidyl choline of liver epithelial cells in culture. The elevated methionine requirements of transformed liver epithelial cells could not be revised by high media levels of purine or pyrimidine nucleosides or by polyamines, indicating a specific requirement for the methyl group of methionine. Causes of elevated methionine requirement of transformed hepatocytes include a dependence upon increased concentrations of exogenous amino acids and diminished capacity to synthesize methionine.

Significance to Biomedical Research and the Program of the Institute: The experiments under this project aim to delineate the roles of choline, methionine, vitamin B_{12} and folic acid in chemical carcinogenesis. The evidence accumulated to date suggests the following partial generalizations: 1) The accelerating effect of low-lipotrope diets on carcinogenesis appears attributable to their affects in carcinogen metabolism. 2) The accelerating affects of vitamin B_{12} on chemical carcinogenesis may be due to the host's synthesis of methionine to meet the demands of the tumor. 3) The lipotrope deficiencies seen in carcinogen-treated or in tumor-bearing animals appears to be a consequence of an elevated requirement for methionine, choline and reduced folates in rapidly proliferating tissue. These dietary factors will be used in the prevention or inhibition of cancer.

Proposed Course of the Project: Carcinogenesis by standard carcinogens in animals fed chemically-defined diets will be determined. The carcinogenicity and metabolism of ethionine in different species will be studied. The effects of methionine on tumor promotion and on the toxicity of endogenous electrophiles is being studied.

Publications

Linnell, J. C., Quadros, E. V., Matthews, D. M., Morris, H. P. and Poirier, L. A.: Altered cobalamin distribution in rat hepatomas and in the livers of rats treated with diethylnitrosamine. Cancer Res. 37: 2975-2978, 1977.

Petri, W. A. and Poirier, L. A.: A methionine-reversible folate deficiency in rats following the acute administration of diethylnitrosamine and ə-naphthylisothiocyanate. Chem.-Biol. Interac. 17: 1-7, 1977.

Wilson, M. J. and Poirier, L. A.: Increased methionine requirements of transformed liver cells in vitro. Exp. Cell Res. 111: 397-400, 1978.

Poirier, L. A., Grantham, P. H. and Rogers, A. E.: The effects of a marginally lipotrope-deficient diet on the hepatic levels of S-adenosylmethionine and on the urinary metabolites of 2-acetylaminofluorene in rats. Cancer Res. 37: 744-748, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04581-03-CMT

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Analytical Applications of Mass Spectrometry to Carcinogenesis Problems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. Roller	Chemist	CMT NCI
OTHER:	L. Keefer	Head, Anal. Chem. Sec.	CMT NCI
	J. Miller	Chemist	CMT NCI
	R. Angeles	Staff Fellow	CMT NCI
	E. Weisburger	Branch Chief	CMT NCI
	P. Grantham	Chemist	CMT NCI
	T. Benjamin	Chemist	CMT NCI
	J. Rice	Head, Perinat. Carc. Sec.	EXP NCI
	H. Gelboin	Branch Chief	CH NCI
	S. Yang	USUHS, Bethesda, Md.	
	A. Aszalos	Litton Bionetics	FCRC
	J. Chan	Litton Bionetics	FCRC

(continued on next page)

COOPERATING UNITS (if any)
Perinatal Carcinogenesis Section, EXP, NCI; Differentiation Control Section, EXP, NCI; Molecular Carcinogenesis Section, CH, NCI; Lung Cancer Branch, NCI

LAB/BRANCH
Carcinogen Metabolism and Toxicology Branch, Carcinogenesis Research Program

SECTION
Analytical Chemistry Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.2	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The laboratory is involved in carrying out independent research, as well as participation in collaborative projects on carcinogenesis related problems, where mass spectrometry, as an analytical tool, can be applied to determine the structure or confirm the identity of non-polymeric organic molecules of interest. Studies include: (1) the identification of carcinogen metabolites in activation and metabolism studies; (2) the identification of naturally occurring potential carcinogens in the environment; (3) elucidation of mass spectral fragmentation mechanisms; (4) development of methods for derivatization and analysis of carcinogens as well as of antitumor agents; (5) analysis of bioactive materials that may play a role in cancer causation and prevention mechanisms, such as vitamin A derivatives and substances with immunological properties.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04581-03-CMT

R. Stroshane	Litton Bionetics	FCRC
M. Sporn	Branch Chief	LC, NCI
C. Frolik	Senior Staff Fellow	LC, NCI
R. Moore	Chem. Dept., U. Hawaii, Honolulu	
M. Goren	Natl. Jewish Hosp., Denver	

Project Description

Objectives: (1) To study in some detail the mass spectra of carcinogens and of some of their possible metabolites, such as aromatic hydrocarbons, aromatic amines and N-nitroso compounds, with the aim of applying this knowledge to develop appropriate analytical methods. (2) To apply the mass spectrometry method for the analysis and identification of metabolites in carcinogen activation and metabolism studies and to elucidate the enzymatic mechanisms of such transformations using deuterium labelled substrates. (3) To elucidate the chemical structure of certain natural bioactive materials, or of their synthetic analogs, that may play a role in cancer causation and prevention mechanisms.

Methods Employed: A high resolution double focusing mass spectrometer system was used, interfaced with a gas chromatograph, and run in the electron impact ionization mode. A number of synthetic and metabolic samples were also prepurified by collaborators using high pressure liquid chromatography. In some cases suitable chemical characterizations were necessary. A number of N-nitroso-C-α-acetoxydialkylnitrosamines were synthesized for mass spectral fragmentation studies.

Major Findings: N-Nitroso-C-α-acetoxydimethylnitrosamine, the acetate ester of the supposed carcinogenic metabolite of N-nitrosodimethylamine, was synthesized earlier.[1] Now, for mass spectral studies 9 analogs were prepared, of general formula R^1-N(NO)-CHR^2-$OCOR^3$. Detailed high resolution studies indicate that these compounds undergo molecular rearrangements upon electron impact to diazonium ions, alkane diazotic acid and to ions corresponding in composition to the acetate of alkyldiazotic acid. The formation of these species may have relevance to the solution chemistry of α-oxygenated dialkylnitrosamines.

The mass spectra of a number of benzo[a]pyrene, benz[a]anthracene and 7,12-dimethylbenz[a]anthracene derivatives are being studied, both of synthetic as well as of metabolic origin, including those labelled with ^{18}O, ^{2}H, and ^{14}C isotopes. In application of these data, in a collaborative effort with the Chemistry Branch of the Division and with S. Yang (USUHS) both the enzymatic metabolism of benzo[a]pyrene in presence of $^{18}O_2$ gas, and the mode of action of epoxide hydratase in $^{18}OH_2$ on synthetic and metabolic epoxide were studied. Mass spectral analysis of the various 4,5-, 7,8-, and 9,10-dihydrodiols and of their acid dehydration products, the phenols, and the measurement of optical activities revealed stereospecific epoxidations and substrate-stereoselective and product-stereospecific hydration of the epoxides to dihydrodiols. Analysis of products in a similar study

of microsomal oxidation of 3-deuterobenzo[a]pyrene and of 4-deuterobenz[a]-anthracene revealed the transient intermediacy of the 2,3-, and of the 3,4-epoxides, respectively, from these two hydrocarbons, even though both epoxides appeared to be too unstable for isolation. For the first time the direct isolation of the metabolic 5,6-epoxide, and of the trans-8,9-diol-10,11-epoxide of benz[a]anthracene was accomplished by HPLC and characterized by spectral techniques.

In collaborative studies within the Branch, in vivo metabolic studies of 2,4-toluenediamine and of 2,4-diaminoanisole in the rat are progressing and a number of interesting metabolites have already been characterized.

Efforts are being continued, in collaboration with L. De Luca, to characterize vitamin A related metabolites from biological sources, and independently with M. Sporn's group to characterize metabolites of retinoic acid. Work with the latter group led to the characterization of 2 monooxygenated metabolites of retinoic acid. In a continuing study with M. B. Goren of the National Jewish Hospital, Denver, recent spectral studies on synthetic multi-acylated trehalose derivatives as model compounds have proved useful in better understanding the spectral behavior of complex trehalose derivatives, isolated from *Mycobacterium tuberculosis*. The natural compounds may have interesting immunological properties and perhaps some tumor suppressive activity.

In a continuing investigation of the chemical components of the edible seaweed *Asparagopsis taxiformis* more than 80 multiply halogenated compounds have been structurally determined, in a collaborative study with R. E. Moore of the University of Hawaii. Most recently identified structures include 3 monohalo-, 4 dihalo-, and 3 trihaloacrylic acids. In some cases, for example in the case of the naturally occurring 3-Z-monobromoacrylic acid, all 3 possible structural isomers were also synthesized and spectral data were compared. A number of these naturally occurring halogen compounds could be viewed as potential carcinogens.

In collaborative efforts with the Fermentation Laboratory, FCRC, on studies of potential chemotherapeutic agents, the characterization of two new antibiotics related to griseorhodin A have been completed and studies on the mass spectral behavior of two *ansa* antibiotics, rifamycin SV and geldanamycin, are continuing.

Significance to Biomedical Research and the Program of the Institute: The understanding of the mass spectral behavior of specific classes of organic compounds is prerequisite to the interpretation of spectra and to the structural determination of a number of biologically important compounds. Toward this goal detailed spectral studies on known N-nitroso compounds, polycyclic aromatic compounds and on macrocyclic antibiotics have been valuable. Structural studies on metabolites of aromatic amines and of poly-

cyclic aromatic hydrocarbons have given insight into the pathways of carcinogen degradation or activation. Identification of carcinogens or of potential carcinogens in man's immediate environment or food staple is important in developing prevention methods.

Proposed Course of Project: The studies described above will be completed and published. A number of metabolic studies are continuing and their significance to cancer causation will be evaluated.

Publications

De Luca, L. M., Frot-Coutaz, J. P., Silverman-Jones, C. S. and Roller, P. P.: Chemical synthesis of phosphorylated retinoids; their mannosyl acceptor activity in rat liver membranes. J. Biol. Chem. 252: 2575-2579, 1977.

Grantham, P. H., Mohan, L., Benjamin, T., Roller, P. P., Miller, J. R., and Weisburger, E. K.: Comparison of the metabolism of 2,4-toluenediamine in rats and mice. J. Toxicol. Environ. Health (in press).

Yang, S. K., Fu, P. P., Roller, P. P., Harvey, R. G. and Gelboin, H. V.: Evidence for an unstable 3,4-epoxide as a metabolic intermediate of benz[a]-anthracene. Fed. Proc. 37: 597, 1978.

Yang, S. K., McCourt, D. W., Gelboin, H. V., Miller, J. R., and Roller, P. P.: Stereochemistry of the hydrolysis products and their acetonides of two stereoisomeric benzo[a]pyrene-7,8-diol-9,10-epoxides. J. Amer. Chem. Soc. 99: 5124-5130, 1977.

Yang, S. K., Roller, P. P., Fu, P. P., Harvey, R. G. and Gelboin, H. V.: Evidence for a 2,3-epoxide as an intermediate in the microsomal metabolism of benzo[a]pyrene to 3-hydroxybenzo[a]pyrene. Biochem. Biophys. Res. Commun. 77: 1176-1182, 1977.

Yang, S. K., Roller, P. P. and Gelboin, H. V.: Enzymatic mechanism of benzo-[a]pyrene conversion of phenols and diols and an improved high-pressure liquid chromatographic separation of benzo[a]pyrene derivatives. Biochemistry 16: 3680-3687, 1977.

Yang, S. K., Roller, P. P. and Gelboin, H. V.: Mechanism of microsomal activation of benzo[a]pyrene to diol epoxides: The separation and characterization of intermediates and products. In Ullrich, V., Roots, I., Hildebrandt, A., Estabrook, R. and Conney, A. H. (Eds.). Microsomes and Drug Oxidations. Oxford, England, Pergamon Press, 1977, pp. 403-410.

Yang, S. K., Roller, P. P. and Gelboin, H. V.: Benzo[a]pyrene metabolism: Mechanism in the formation of epoxides, phenols, dihydrodiols, and the 7,8-diol-9,10-epoxides. In Jones, P. W. and Freudenthal, R. I. (Eds.). Carcinogenesis, Vol. 3, Polycyclic Aromatic Hydrocarbons. New York, Raven Press, 1978, pp. 285-301.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04582-03-CMT

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Structure Activity Correlations in Carcinogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Lionel A. Poirier — Head, Nutrition & Metabolism Section — CMT NCI
OTHER: None

COOPERATING UNITS (if any)
Dr. M. Shimkin, University of California, La Jolla
Dr. C. T. Helmes, SRI International, Menlo Park, California
Dr. V. Simmon, SRI International, Menlo Park, California

LAB/BRANCH
Carcinogen Metabolism & Toxicology Branch, Carcinogenesis Research Program

SECTION
Nutrition & Metabolism Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The mutagenic and carcinogenic activities of previously untested electrophilic agents were investigated. *Salmonella* TA100 was used in standard plate tests to determine the mutagenic activities of the alkyl halides. The ability of alkyl halides to produce lung adenomas in strain A mice was also studied. In general, branching and ease of dissociation increased both the mutagenic and carcinogenic activities of the alkyl halides. Nucleophilic substitution tended to increase the toxicity, the mutagenicity and the tumorigenic activity of the alkyl halides.

PHS-6040
(Rev. 10-76)

Project No. Z01-04582-03-CMT

Project Description

Objectives: The accumulation of evidence has indicated that the activated form of most, if not all, chemical carcinogens consists of a reactive electrophile. The aim of these studies is to determine the extent to which the electrophilic hypothesis of carcinogenesis may be used to predict the potential carcinogenic activities of unknown compounds. Mutagenesis towards microbial systems in vitro is used to obtain a rough measure of a compound's ability to produce reactive intermediates. Guidelines for assessment of the carcinogenic potential of a chemical are developed from a knowledge of its chemical reactivity, metabolic pathways and mutagenic activity.

Methods Employed: The carcinogenic activities of previously untested compounds are tested by standard protocols, generally by the production of mouse lung adenomas in strain A mice. Mutagenicity studies are being performed with the standard and modified Ames assay with *Salmonella*, with polymerase A-deficient *E. coli* and with *E. coli* WP2(hcr-).

Major Findings: The mutagenic and carcinogenic activities of several alkyl halides have been determined. Virtually all tested compounds are mutagenic to one or more of the tester strains employed. In general, branching, ease of ionization, and nucleophilic substitution which increase the electrophilic reactivity of these agents, also tended to increase their mutagenic, toxic, and tumorigenic activities. Nucleophilic substitution into alkyl bromides often made them too toxic to be tested in the mouse lung adenoma assay.

Significance to Biomedical Research and the Program of the Institute: The aim of these studies is to increase the base of theoretical knowledge by which the potential carcinogenic hazards to man of previously untested carcinogens can be estimated. The evidence accumulated to date indicates that among the alkyl halides, chemical reactivity is a reasonable guide to biologic activity.

Proposed Course of Project: Other classes of electrophilic agents will be tested for their carcinogenic and mutagenic activities. The role of anchimeric assistance in the carcinogenic and mutagenic activities of the alkyl chlorides will be further examined.

Publications

Poirier, L. A. and Helmes, C. T.: Carcinogenesis by esters and benzylic alcohols. In Asher, I. and Zervos, C. (Eds.). Proceedings of the 2nd FDA Office of Science Summer Symposium, Washington, D. C., U. S. Government Printing Office, (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04617-13-CMT

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Carcinogen Screening Operations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. K. Weisburger	Chief	CMT NCI
OTHER:	T. Benjamin	Chemist	CMT NCI
	C. A. Brown	Microbiologist	CMT NCI

COOPERATING UNITS (if any) Dr. M. B. Shimkin, University of California, La Jolla; Dr.B. Ulland, Hazleton Labs., Virginia; Dr. M. Wenk, Microbiological Associates, Inc., Bethesda, Md.; Dr. A. B. Russfield and Dr. A. S. Krishnamurthy, Mason Research Institute, Worcester, Mass.

LAB/BRANCH
Carcinogen Metabolism and Toxicology Branch, Carcinogenesis Research Program

SECTION

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NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The chronic effects of environmental chemicals or of mixtures of chemicals are determined in suitable animal models. Factors influencing the outcome such as sex, strain, and species of animal are investigated.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04617-13-CMT

Project Description

Objectives: Responsibilities are assumed on the design, performance and evaluation of studies in either contract or intramural laboratories, on the chronic effect and carcinogenicity of chemicals or of mixtures of chemicals. The aim of these investigations is to gather information on hazards involved in handling certain chemicals or drugs, and also on the mechanisms underlying joint effects in mixtures. Of particular interest are such mixtures which tend to give increased or decreased overall carcinogenic effect. Of concern also is the methodology of carcinogen screening, with emphasis on improvements in sensitivity, speed and economy. Short-term assays are under development, taking into account the need to secure biochemical activation of most environmental agents. Guidelines for assessment of the carcinogenic potential of chemicals are developed, by consideration of their chemical structures, metabolic pathways and mutagenic activity.

Methods Employed: Attempts are made to secure information on chemicals representing the greatest hazard to the largest number of people. Chemicals and drugs are rated as to priority on this basis as well as other criteria such as relationship to known carcinogens, epidemiologic observations, indications of specific toxicity and related factors. Standard protocols as well as newly designed protocols attempting to increase the sensitivity and speed of such tests are utilized. The carcinogenc and mutagenic activities of various compounds are determined in appropriate systems, either directly or after metabolic activation. Exploratory meetings with relevant national and international organizations in industry, in government and in university environments are setting the stage for a concerted development effort in this area.

Major Findings: Industrial or environmental chemicals. An extensive paper on the long-term effects of many aromatic amines used as dyestuff intermediates or starting materials for industrial processes has been prepared. In many cases the results from these studies are the only ones available for these important compounds.

Significance to Biomedical Research and the Program of the Institute: Many substances in the environment have not yet been evaluated for chronic toxicity and possible carcinogenicity. It is important to secure such information after deliberate and judicious setting of priorities. Cancer in man has been observed in the past as a result of exposure to chemicals subsequently demonstrated to be carcinogenic. The aim of this program is to forestall such unintentional exposures of man which would result in neoplastic disease in the future.

Proposed Course of Project: Continuing efforts are required 1) to assess relative priorities of materials to be tested, 2) to undertake studies on variations in the protocols to simplify or to develop novel test techniques which are more sensitive, more specific and more economic, and 3) to apply such tests to identify carcinogenic hazards in the environment.

Publications

Theiss, J. C., Stoner, G. D., Shimkin, M. B., and Weisburger, E. K.: Test for carcinogenicity of organic contaminants of U. S. drinking waters by pulmonary tumor response in strain A mice. Cancer Res. 37: 2717-2720, 1977.

Weisburger, E. K.: Bioassay program for carcinogenic hazards of cancer chemotherapeutic agents. Cancer 40: 1935-1949, 1977.

Weisburger, E. K.: Carcinogenic natural products. Proceedings of the FDA Meeting on Structural Correlates of Carcinogenesis and Mutagenesis: A Guide to Testing Priorities? (in press).

Weisburger, E. K.: Carcinogenicity studies on halogenated hydrocarbons. Environ. Health Perspectives (in press).

Weisburger, E. K.: Chemical carcinogenesis. In Toxicology for Chemists. (in press).

Weisburger, E. K.: Foreword. In A Rational Evaluation of Pesticidal Versus Mutagenic/Carcinogenic Action. GPO-NIH (in press).

Weisburger, E. K.: History of the Journal of the National Cancer Institute. J. Nat. Cancer Inst. 59: 601-604, 1977.

Weisburger, E. K.: Industrial and environmental cancer risks. In Sax, N.I. (ed.). Dangerous Properties of Industrial Materials. (in press).

Weisburger, E. K.: Structure-activity relationships in carcinogenic drugs. In Bungaard, H., Jull, P. and Kofod, H. (eds.). Alfred Benzon Symposium X. Drug Design and Adverse Reactions. Denmark, Munksgaard, 1977, pp. 246-260.

Weisburger, E. K., Russfield, A. B., Homburger, F., Weisburger, J. H., Boger, E., Van Dongen, C. G., and Chu, K. C.: Testing of 21 environmental aromatic amines or derivatives for long-term toxicity or carcinogenicity. J. Toxicol. Environ. Health (in press).

Weisburger, E. K.: Carcinogenic natural products. In Carcinogenic Natural Products (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04618-13-CMT

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Endogenous and Exogenous Factors in Chemical Carcinogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. P. Evarts	Veterinary Medical Officer	CMT NCI
OTHER:	C. A. Brown	Microbiologist	CMT NCI
	M. H. Mostafa	Visiting Associate	CMT NCI
	E. K. Weisburger	Chief	CMT NCI

COOPERATING UNITS (if any)

Dr. C. Michejda, FCRC; Dr. B. Cockrell, Experimental Pathology Laboratories

LAB/BRANCH

Carcinogen Metabolism and Toxicology Branch, Carcinogenesis Research Program

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.3	2.3	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effects structural alterations may have on the carcinogenicity or toxicity of various compounds are determined. Methods to inhibit the action of known carcinogens by administration of other compounds are devised. The biochemical, morphological, and physiological bases for such inhibitory effects are studied.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04618-13-CMT

Project Description

Objectives: Investigations to discover the intimate factors involved in the initiation and development of neoplasia are explored by means of animal experiments in various species. The studies utilized a number of chemical carcinogens administered to animals under varying conditions of diet, endocrine situation or immunological status so as to gain insight into controlling mechanisms in the initiation and development of chemically induced tumors. Further efforts deal with the development of systems which lead to the prevention of the processes of chemical carcinogenesis and a study of the mechanisms related thereto.

Methods Employed: Chemical carcinogens or unknown agents to be evaluated for carcinogenicity are administered by a number of routes to several strains of rats or mice on specific dietary regimens. Additionally, certain chemicals with specifically tailored structures or properties are given together with chemical carcinogens to determine whether tumor induction can be delayed or inhibited. Host factors such as the endocrine or immunologic status are being investigated in relation to the effectiveness of tumor formation. After judiciously selected treatment and observation periods, autopsies of the experimental animals are performed. Upon histologic processing of tissues, the results in experimental systems are carefully evaluated in relation to control groups.

Major Findings: (1) Dimethylnitrosamine (DMN) demethylase activity of rat liver microsomes after partial hepatectomy. DMN-Demethylase is the primary controlling factor in the metabolism of DMN to both toxic and carcinogenic metabolites. To investigate why partial hepatectomy increases the hepatocarcinogenicity of DMN, the activity of liver DMN demethylase was determined on Wistar male rats at different time intervals after partial hepatectomy. The enzyme activity reached its lowest point (47%) one day after operation, and at three days had recovered to about 90% of the control values. The LD_{50} for partially hepatectomized rats 44 hours after i. p. injection of DMN was 114 mg/kg body weight as compared to 82 mg/kg for control animals. The reduction rather than increase of enzyme activity following hepatectomy shows that increased DMN carcinogenicity following hepatectomy is not caused by a compensatory increase of demethylase activity associated with liver regeneration.

(2) In vitro effect of L-tryptophan and its metabolites on dimethylaminoazobenzene (DAB) reductase activity of rat liver. An earlier study from this laboratory showed that L-tryptophan protected against 3'-methyl-4-dimethylaminoazobenzene-induced liver tumors. Azoreductase is a flavoprotein enzyme which cleaves the azo linkage one of the metabolic detoxification reactions toward the carcinogenic effect of DAB. Therefore the in vitro effect of L-tryptophan and its metabolites L-kynurenine, anthranilic acid, kynurenic acid, quinaldic acid, 3-hydroxy-DL-kynurenine, 3-hydroxyanthranilic acid, xanthurenic acid, quinolinic acid, N-methylnicotinamide, N'-methylnicotinamine and cinnabarinic acid on rat liver azo reductase activity were

determined. Three of these metabolites, 3-hydroxykynurenine, 3-hydroxy-anthranilic acid and cinnabarinic acid decreased enzyme activity. The inhibition was greater if the buffered solutions of 3-hydroxykynurenine and 3-hydroxyanthranilic acid were kept overnight before use, but the effect was prevented if 3-hydroxykynurenine and 3-hydroxyanthranilic acid were prepared in solutions of L-ascorbic acid and/or L-cysteine HCl. This observation indicates that the autoxidation products, which are phenoxazine compounds, were actually responsible for inhibition of the enzyme.

(3) The effect of L-tryptophan and certain other amino acids on liver dimethylnitrosamine demethylase activity. Our earlier study showed that L-tryptophan had a protective effect and reduced the number of anaplastic tumors induced by diethylnitrosamine and by 3'-methyl-4-dimethylaminoazo-benzene. The liver mixed function oxidase system plays an important role in the metabolism of carcinogens. The present study was to investigate the effect of L-tryptophan on liver DMN-demethylase, one of the mixed function oxidase enzymes. Other amino acids of different chemical structures were included in this study, but only L-tryptophan increased the enzyme activity. The increase was dependent on the age of the animals, being most prominent at 30 days of age, or on the length of the feeding period.

(4) Effect of sulfaguanidine on carbon tetrachloride (CCl_4) induced lesions of rat liver. A project was undertaken to determine whether sulfaguanidine would protect rats from the hepatocarcinogenicity of carbon tetrachloride since it protected mice from acute toxic effects. The rats were injected with carbon tetrachloride and also fed diets supplemented with 2% sulfa-guanidine. Although liver tumors were not produced, carbon tetrachloride did cause cirrhosis, adenofibrosis and foci of cellular alterations, some of which are considered preneoplastic. Sulfaguanidine did give some protection against the toxic effects of carbon tetrachloride. Animals prefed with sulfaguanidine and also receiving it simultaneously with carbon tetra-chloride showed the greatest amount of protection. Electron microscopic analysis of livers showed the endoplasmic reticulum in the sulfaguanidine rats to be far less damaged and disorganized than the livers of those receiving carbon tetrachloride alone.

Significance to Biomedical Research and the Program of the Institute: The prevention and cure of neoplasia hinges on an understanding of the intimate factors involved in the pathogenesis of cancer. The research performed within the framework of this report aims to produce data which will permit the comprehension of the underlying mechanisms involved in chemical carcinogenesis.

Proposed Course of Project: Emphasis will be placed on methods for inhibiting the carcinogenic process and on understanding the various steps leading to such inhibitions. Thus studies will include variations in endogenous factors as well as select exogenous materials.

Publications

Evarts, R. P.: Effect of L-trytophan on diethylnitrosamine and 3'-methyl-4-N-dimethylaminoazobenzene hepatocarcinogenesis. Fd. Cosmet. Toxicol. 15: 431-435, 1977.

Evarts, R. P. and Brown, C. A.: Morphology of mammary gland, ovaries and pituitary gland of hydroxylamine fed C3H/HeN mice. Lab. Invest. 37: 53-63, 1977.

Evarts, R. P. and Mostafa, M. H.: Dimethylnitrosamine demethylase activity of the rat liver microsomes after partial hepatectomy. Biochem. Pharmacol. (in press).

Evarts, R. P., Brown, C. A., and Atta, G. J.: The effect of hydroxylamine on the morphology of the rat mammary gland and on the induction of mammary tumors by 7,12-dimethyl(a)anthracene. Lab. Invest. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04619-13-CMT

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Studies on the Metabolism of Chemical Carcinogens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. H. Grantham	Chemist	CMT NCI
OTHER:	L. M. Tahan	Chemist	CMT NCI
	T. Benjamin	Chemist	CMT NCI
	T. V. Reddy	Visiting Fellow	CMT NCI
	R. Ramanathan	Visiting Associate	CMT NCI
	P. P. Roller	Chemist	CMT NCI
	E. K. Weisburger	Chief	CMT NCI

COOPERATING UNITS (if any)
Analytical Chemistry Section, CMT, NCI
Nutrition and Metabolism Section, CMT, NCI

LAB/BRANCH
Carcinogen Metabolism and Toxicology Branch, Carcinogenesis Research Program

SECTION

INSTITUTE AND LOCATION
NCI,NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The metabolic pathways of chemical carcinogens, generally various aromatic amines, are studied both in animals or in isolated tissues. Metabolites are separated by various techniques such as thin-layer, column, gas-liquid or high pressure liquid chromatography. Identification is made through physico-chemical means such as mass spectrometry, nuclear magnetic resonance and ultraviolet spectra. Interaction of metabolites with cellular constituents is determined. The effect inhibitors or promoters of the carcinogens exhibit on the metabolic patterns is studied.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04619-13-CMT

Project Description

Objectives: The aim of this research is to gather data relevant to the etiology of neoplasia at the molecular level. To this end chemical carcinogens, especially those of the aromatic amine type, are being utilized. Their metabolism and interaction with host tissues and specific targets are studied. Simplified yet realistic model systems are devised in order to gain an understanding of the fundamental events operating in chemical carcinogenesis. Specific inhibitors and accelerators of the carcinogenic process are applied as tools to develop information on and permit discrimination between biochemical events directly involved in the neoplastic change and those representing other reactions.

Methods Employed: Biochemical and pharmacological techniques are applied to determine the metabolism of N-hydroxy-N-2-fluorenylacetamide and related compounds in various animal species. This includes study of the enzyme systems concerned with certain metabolic steps. Procedures are developed and applied for the separation, purification and analysis of macromolecular constituents such as DNA, RNA and proteins from tissues of animals treated with chemical carcinogens and from control animals. The metabolism of chemical carcinogens and related compounds, and interaction with host targets are examined in vivo and in vitro in the presence of various chemicals or antibiotics which affect the carcinogenic process.

Major Findings: (1) Continuation of study on metabolism of 2,4-diaminoanisole. A ring hydroxylated metabolite with a mass of 238 and a presumed N-hydroxylated metabolite have been isolated from urines of animals fed 2,4-diaminoanisole. NMR studies will be run on these compounds to decide more definitely whether the hydroxy group is on the ring or on the nitrogen. Preliminary experiments with ^{14}C-2,4-diacetylaminoanisole have shown that the major metabolites are 2,4-diacetylaminoanisole, amounting to about 15% of the dose, and 4-acetylamino-2-aminoanisole, amounting to about 5% of the dose, plus an unknown which gave 4-aminoacetylanisole to the extent of about 7-1/2% of the dose when it was exposed to light on thin layer plates. On the other hand when 4-acetylamino-2-aminoanisole was given to rats the major metabolites in the free fraction included 2,4-diacetylaminoanisole, 4-acetylamino-2-aminoanisole, and 2,4-diacetylaminophenol plus an unknown hydroxylated compound. When 2-acetylamino-4-aminoanisole was given to rats the major metabolites in the free fraction were 2,4-diacetyl- and 4-acetylamino-2-aminoanisole. However, no 2-acetyl-4-aminoanisole was observed indicating that there is preferential acetylation of the 4-amino group in 2,4-diaminoanisole. However, none of these studies showed a larger fraction of a possible N-hydroxylated metabolite than during the studies with 2,4-diaminoanisole. Another possible metabolite with a mass of 236 is being investigated.

(2). Investigation of formyl derivative of 2,4-diaminoanisole. In earlier studies it was found that a formyl derivative was present in the free fraction of ethyl acetate extracts of urines of 2,4-diaminoanisole treated

rats. However, this compound was found to be an artifact due to the presence of some contamination in the ethyl acetate used previously. When a different brand of ethyl acetate or another solvent system was used for extraction the formyl derivative was not observed. In addition when the synthetic compound 4-acetylamino-2-aminoanisole was dissolved in the original brand of ethyl acetate and evaporated to dryness under nitrogen, high pressure liquid chromatography indicated the presence of the formyl derivative. When the compound was dissolved in the second brand of ethyl acetate and treated as above there was no indication of a formyl derivative.

(3) Effect of 2-aminoanthraquinone on lipid metabolism. Various studies have shown that 2-aminoanthraquinone (2-AAQ) was nephrotoxic and carcinogenic when fed to rats. 2-AAQ and its formyl and acetyl derivatives have been isolated from the kidneys of rats fed 2-AAQ. Accumulation of 2-AAQ in the kidney suggests a possible defective membrane permeability. As phospholipids have been strongly implicated in drug metabolism and membrane structure, the present study was designed to investigate the possible role of phospholipids in the transport of 2-AAQ and also its ability to maintain the structural integrity of the drug metabolizing enzymes. However, in male and female rats (F344) on a 2% AAQ diet, there was no significant change in the total phospholipids of liver in female rats compared to the controls after 8 weeks of feeding. By the end of 14 weeks there was an increase of 40% above the control levels. In the case of male rats the maximal changes (80-85% over controls) were obtained by the 8 week period with no further change at the end of 14 weeks. Further analyses of the phospholipid changes of the mitochondria and microsomes from 2-AAQ-fed rats are in progress.

(4) Butylnitrosourea and lipid metabolism. Preliminary results suggested that in male C57Bl mice, a single i.p. injection of butylnitrosourea produced intestinal tumors and pronounced changes in the lipids of liver, brain, kidney and adipose tissues. This aspect is being investigated in detail.

(5) Microsomal metabolism of 2,4-toluenediamine (2,4-TDA). To understand the patterns of metabolic degradation of 2,4-TDA to reactive intermediates capable of interacting with macromolecules and to non-reactive conjugated metabolites, the metabolism of 2,4-TDA was studied in vitro with purified microsomes. The microsomes were prepared from rats treated with the inducers phenobarbital and Aroclor 1254 prior to sacrifice.

In the initial experiments the metabolites were extracted with water-saturated ethyl acetate. Since degradation of 2,4-TDA in the control incubations was observed during extraction with ethyl acetate, a system of $CHCl_3$/propanol was established for extraction. Thin layer chromatography of the $CHCl_3$/propanol extract showed the presence of three major metabolites. The aqueous layer after final extraction with $CHCl_3$/propanol contained about 7% of the total radioactivity; only 50% of the radioactivity

of the aqueous layer could be solubilized by arylsulfatase and beta-glucuronidase to forms extractable with $CHCl_3$/propanol. This suggests that about 7% of the total metabolites in the microsomal metabolism of 2,4-TDA existed as conjugates.

To minimize the degradation of metabolites they were derivatized by both methylation and acetylation. They were further resolved by high performance liquid chromatography for suitable identification by UV spectroscopy and confirmation by mass spectrometry. Initial experiments done on the binding of the microsomal metabolic intermediates of 2,4-TDA to endogenous microsomal protein and RNA indicated that the specific activity of binding to RNA was always greater than protein at all times of incubation and at different protein concentrations. The capacity of the extrahepatic tissues like kidney and testis to metabolize 2,4-TDA is also being carried out.

Significance to Biomedical Research and the Program of the Institute: By the utilization of certain chemical carcinogens the molecular mechanism of steps leading to cancer is being explored. If the entire process is viewed as a series of steps from the primary interaction between an agent and cellular constituents, followed by multiplication of abnormal cells which in turn can undergo further transformations, there are a number of points where one could prevent or even reverse such interactions. Thus, the ultimate aim of these studies is to comprehend fully the sequence of complex reactions leading to cancer and eventually to be in a position to prevent them under realistic conditions in man.

Proposed Course of Project: The program of elucidating the biochemistry of carcinogenesis, our eventual goal, is a continuing, long-term effort. Certain promising leads which have been discussed in this account will be followed up in further experiments.

Publications

Gothoskar, S. V., Benjamin, T., Roller, P. P., and Weisburger, E. K.: Metabolic fate of 2-aminoanthraquinone, a probable hepatocarcinogen and a nephrotoxic agent in the Fischer rat. Proceedings of the Third International Symposium on the Detection and Prevention of Cancer. New York, Marcel Dekker, Inc. (in press).

Grantham, P. H., Mohan, L., Benjamin, T., Roller, P. P., Miller, J. R. and Weisburger, E. K.: Comparison of the metabolism of 2,4-toluenediamine in rats and mice. J. Toxicol. Environ. Health (in press).

Weisburger, E. K.: Mechanisms of chemical carcinogenesis. Ann. Rev. Pharmacol. Toxicol. (in press).

Weisburger, E. K.: Species specific biochemical pathways of malignant growth. In Pathologic Growth in Plants, Animal, and Man. Kaiser, H. E. (ed.). Johns Hopkins University Press, Baltimore (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04620-13-CMT

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Mode of Action of Chemical Carcinogens - Chemical Investigations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Benjamin	Chemist	CMT NCI
OTHER:	E. K. Weisburger	Chief	CMT NCI

COOPERATING UNITS (if any)
Analytical Chemistry Section, CMT, NCI
Dr. Robert C. Atkins, James Madison University, Harrisonburg, Virginia

LAB/BRANCH
Carcinogen Metabolism and Toxicology Branch, Carcinogenesis Research Program

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.3	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project serves as a supporting function to the studies on metabolism of various toxic or carcinogenic agents in this Branch. The purity of compounds being investigated for toxicity or carcinogenicity is determined along with pertinent physical characteristics, useful in the design of the experiments. Possible metabolites of various aromatic amines, such as 2,4-toluenediamine, 2,4-diaminoanisole, and 2-aminoanthraquinone are synthesized as reference materials for metabolism studies. Methods for separation of aromatic amines and their metabolites are investigated.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04620-13-CMT

Project Description

Objectives: In order to gain an understanding of the etiology of cancer, chemical studies are performed on the properties of certain carcinogens. Model studies on the reactions of chemical carcinogens with potential targets are performed.

Methods Employed: Properties of chemicals acquired or synthesized for the small scale bioassay of chemical carcinogens are investigated to determine the purity of these materials. In model experiments the reactions of certain of these agents, particularly their primary and ultimate reactive forms, with select potential targets are studied. The materials produced are made available for related studies on the biochemistry and biology of the carcinogenic process. Methods for purification and analysis of various chemicals are applied.

Major Findings: During the past year, in addition to synthesizing reference materials or checking the purity of compounds for various small scale studies, ^{14}C-labeled 2,4-diacetylaminoanisole was prepared for a study on the metabolism of some of the intermediates isolated as primary metabolites of 2,4-diaminoanisole. In addition the solubilities of 2-aminoanthraquinone, the N-acetyl, and the N-formyl derivative were determined.

Significance to Biomedical Research and the Program of the Institute: The studies performed are a necessary adjunct to the broad programs on the mechanism of action of chemical carcinogens pursued in this Branch.

Proposed Course of Project: This project will continue along the same lines as previously.

Publications

Weisburger, E. K., Grantham, P. H. and Benjamin, T.: Separation and analysis of various carcinogenic aromatic amines and their metabolites. Proceedings of the Third International Symposium on the Detection and Prevention of Cancer. New York, Marcel Dekker, Inc. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04680-08-CMT

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Development and Application of In Vitro Systems Involving Epithelial Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. J. Wilson	Chemist	CMT NCI
OTHER:	J. Brown	Biological Laboratory Technician	CMT NCI

COOPERATING UNITS (if any)

Dr. E. K. Weisburger,	Chief	CMT NCI
Dr. Ann Kaplan	Chemist	CMT NCI

LAB/BRANCH
Carcinogen Metabolism and Toxicology Branch, Carcinogenesis Research Program

SECTION
Nutrition and Metabolism Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Epithelial-like cells derived from livers of infant Fischer rats are maintained in culture, either continuous or after frozen storage. Ultrastructural alterations produced by long term culture and frozen storage are being examined. Transformation of the cells to a malignant form by various chemical carcinogens is being studied.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04680-08-CMT

Project Description

Objectives: Successful culture of epithelial-like cells derived from livers of 8 to 10 day old Fischer strain 344 rats and malignant transformation of such cultured cells by a variety of chemical carcinogens were discussed in previous reports. Since neoplasia in man and animals involves mainly epithelial cells, our principal aim in current investigations has been to further determine the potential of in vitro epithelial systems for use in rapid bioassay procedures for chemical carcinogens and in examining the biochemical mechanisms of carcinogenesis.

Two problems encountered in past investigations were the relatively long time required to develop cultures that were proliferating adequately to provide enough cells for biochemical investigations and the fact that some sublines of control cells underwent spontaneous malignant transformation as demonstrated by tumor formation following injection of cells into syngeneic hosts. Unfortunately, no distinctive morphological changes were apparent in the cultured cells that induced tumors. In addition, the spontaneous transformation was not correlated with a specific time in culture nor with the number of times a line had undergone subculturing. Therefore, we have emphasized the development of methods for producing a greater number of cultures with similar characteristics so that studies can be repeated as required in more replicable systems. Investigation of pertinent biochemical properties of untreated or chemically exposed cultured cells in respect to the effects of various cultural conditions and in comparison to the characteristics of liver tissue from whole animals should help assess the value of this in vitro system.

Methods Employed: Methods for selecting rat liver cells for culture continue to be developed in this laboratory. This involves detaching and transferring islands of epithelial cells, both mechanically and by trypsinization from cloning cylinders, so that many homogeneous sublines can be cultured from one primary culture.

Previous studies in this laboratory have shown that frozen storage of cells did not alter enzyme levels or cause transformation. To further investigate the effects of frozen storage as well as long term continuous culture on the ultrastructure of rat liver cells, electron microscopy studies have been initiated.

To investigate the potential carcinogenicity of 2-aminoanthraquinone, rat liver epithelial cells were exposed to AAQ for various time periods and at several concentrations.

Major Findings: (1) Chemical transformation of rat liver cells with 2-aminoanthraquinone. Preliminary results indicated transformation of rat liver epithelial cells exposed to 2-aminoanthraquinone for three months. This study has been repeated and the treated cells have been injected subcutaneously into syngeneic hosts to determine whether or not transformation has occurred.

Significance to Biomedical Research and the Program of the Institute: An assay system which can be designed to give results more rapidly, specifically, and reliably than the customary long-term tests for carcinogenicity is highly desirable with the dual purpose of studying fundamental phenomena and the detection of harmful agents in our environment. Full exploitation of workable in vitro systems has not been achieved. It is our goal to develop such systems; an additional advantage of the in vitro systems is that eventually they can established utilizing cells from man. Hence carcinogenesis in vitro with human cells avoids the question of species difference, so often raised in evaluating the significance of carcinogen bioassay systems.

Proposed Course of Project: These epithelial cells will be carefully examined for suitablity as a carcinogen and promoter screening system. Biochemical properties of both normal and transformed cells will be investigated with effort towards establishing biochemical markers of transformation. Ultrastructural properties of both normal and transformed cells will be determined.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04688-08-CMT

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Investigations on Systems Leading to Cancer in the Colon and Small Intestine

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. K. Weisburger	Chief	CMT NCI
OTHER:	C. A. Brown	Microbiologist	CMT NCI
	R. P. Evarts	Veterinary Medical Officer	CMT NCI
	T. Benjamin	Chemist	CMT NCI

COOPERATING UNITS (if any)
Dr. M. L. Wenk, Microbiological Associates, Inc., Bethesda, Md.

LAB/BRANCH
Carcinogen Metabolism and Toxicology Branch, Carcinogenesis Research Program

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.2	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Animals are injected with chemical carcinogens known to induce tumors of the colon and small intestine. Alteration of these tumorigenic effects of various dietary constituents such as proteins, fats, carbohydrates, antioxidants, or bulk material is investigated. Characterization of some of the enzymes as well as their levels in the induced tumors is performed.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04688-08-CMT

Project Description

Objectives: In the United States and Western Europe the mortality rate due to colon and rectum cancer in males is second only to lung cancer, and in females second to breast cancer. In incidence, colon cancer is third after skin and lung in men, and in women, after breast and uterus. However, in other countries such as Japan and Africa, colon cancer incidence is much lower, thus suggesting that environmental factors are associated with the etiology of this important disease. Within recent years several new agents were discovered by which colon cancer could be induced in animals. The goal of the studies to be described is to evaluate how select environmental factors affect the production of cancer of the colon in animals thereby gaining insight into conditions possibly affecting humans.

Methods Employed: Rats, mice and other rodent species were treated with chemicals known to lead to colon cancer, such as 1,2-dimethylhydrazine and azoxymethane. The animals are placed on diets mimicking those utilized in various parts of the world with regard to quantity and quality of protein, fat, carbohydrates, micronutrients, and the amount of bulk material such as cellulose in the diet. After appropriate periods of time the animals are carefully autopsied and the lesions are examined microscopically.

Major Findings: Papers have appeared on various aspects of research with the colon carcinogen azoxymethane.

Significance to Biomedical Research and the Program of the Institute: Establishment of a colon cancer model with similarities to the human disease was necessary to study the effects of diet on colon cancer induction since dietary factors are thought to be responsible for the occurrence of human colon cancer. Our studies suggest that under our experimental conditions, diets containing nutrients thought to be important for the high incidence of human colon cancer in some parts of the world did not increase the incidence of chemically induced cancer. The limitations of this model are obvious.

Proposed Course of Project: New projects will be initiated when more facilities are available.

Publications

Brown, C. A.: The cytochemical demonstration of beta-glucuronidase in colon neoplasms of rats exposed to azoxymethane. J. Histochem. Cytochem. 26: 22-27, 1978.

Weisburger, E. K., Evarts, R. P. and Wenk, M. L.: Inhibitory effect of butylated hydroxyltoluene (BHT) on intestinal carcinogenesis in rats by azoxymethane. Food Cosmet Toxicol. 15: 139-141, 1977.

SUMMARY REPORT

CHEMISTRY BRANCH

October 1, 1977 through September 30, 1978

"Plans, develops, and conducts a research program designed to (1) clarify the molecular biology of carcinogenesis; (2) elucidate the fundamental nature of the interactions of carcinogenic agents, especially chemical, with biological systems in the induction of cancer; (3) define those environmental and endogenous factors which relate to and modify the carcinogenic process; and (4) clarify the metabolic regulatory processes which are related to carcinogenesis."

The goal of the Chemistry Branch is to understand the molecular basis of carcinogenesis with the view toward identifying susceptible populations and preventing human cancer. The research program is designed to understand the molecular basis by which carcinogenic agents cause malignant transformation and identify and characterize those exogenous and endogenous factors involved in carcinogenesis. The Branch seeks to clarify the metabolic interaction of exogenous and endogenous agents in the living organism at the molecular, cellular and organism levels and seeks to understand the consequences of these interactions in terms of cell regulation and carcinogenesis. These processes are studied in biological preparations and cells from experimental animals and humans.

Cancer is a disease in which the genetic information and/or expression is altered. This altered phenotypic expression may be due to genetic or epigenetic events induced by either xenobiotic or endogenous factors which result in altered patterns of gene control. The central aim of the Branch is to understand how exogenous carcinogens and endogenous factors are processed by enzymatic mechanisms, how the carcinogen is converted to active forms and the nature of the interaction between the latter and the gene action system. Systems involved in the repair of genetic damage induced by carcinogens are studied. Understanding is sought on how the interaction with carcinogens results in the modifications which characterize malignant transformation. The aim of these studies is modification, elimination or prophylaxis of the carcinogenic response in the human population.

Senior staff of the Branch is also responsible for the scientific direction of the Chemistry and Molecular Carcinogenesis Segment.

Cell Growth Regulation Section - *"Studies (1) the biochemical factors controlling cell growth and differentiation and the nature of the mechanisms governing the phenotypic expression of genetic information; (2) chemical, physical, and biological agents capable of inducing alterations in growth behavior and differentiation."* The primary goal of this Section is to define the molecular events of malignant transformation induced by chemical carcinogens, and in particular the nature of the molecules responsible for converting a normal cell to its malignant form.

Four major areas of investigation were underway in this Section during the past year.

The development of a system for assay of potent carcinogens using cultured cells. As a continuation of our previous success in the transformation of human cells, quantitation and earlier detection of the transformation of human cells after treatment with chemical carcinogens have been attempted using a promotor, phorbol ester, and the soft agar method. Abortive or transient transformation was observed about one month after chemical treatment. Treatment with promotor enhanced the frequency and shortened the time of the development of abortive transformation, but did not result in the enhancement of stable and malignant transformation,

A quantitative system for assay of carcinogens using subclones derived from BALB/3T3 mouse cells has been improved in the technical aspects and these improvements have been shared with many laboratories in the world that were trying to establish a quantitative assay system for environmental carcinogens.

The study of the mechanism of chemical carcinogenesis by defining genetic, biochemical and physiological factors affecting carcinogenesis is another major area of study. The study of the relation of these factors to the etiological aspects in the incidences of human cancer is included.

One approach taken in this direction is to study DNA repair in human cancer-prone genetic diseases with emphasis on xeroderma pigmentosum (XP) and ataxia telangiectasia (AT). Cells from patients with XP and AT, two autosomal recessive diseases associated with high incidence of neoplasia, have been found to be very sensitive to radiation and chemical carcinogens and to have defective DNA repair. A detailed comparison of the clinical features and cellular abnormalities in AT and XP showed remarkable similarities, particularly in the neurological abnormalities in both diseases. It is suggested that features common to both diseases may be the end result of extremely defective DNA repair in certain cell types. Studies initiated include the determination of existence of genetic heterogeneity in AT cells similar to that in XP cells using the cell fusion method, detection of the carrier state of XP or AT _in vitro_ using haploid (sperm) cells and cell fusion method, determination of chromatin localization of DNA repair in normal and repair of deficient human cells, and repair of DNA damage (strand breaks and release of bases) caused by the chemotherapeutic agent, bleomycin, in normal and repair defective human cell.

It was found that XP cells were not very sensitive to malignant transformation by 4-nitroquinoline-1-oxide when the frequency of transformation was calculated on the basis of the number of cells treated, whereas XP cells were extremely sensitive to the cytotoxic effects of various carcinogens. The comparative study of the transformation frequency per surviving cells after chemical treatment between normal and XP cells is being continued.

Another approach taken to define genetic and other factors affecting chemical carcinogenesis was to clarify the nature of cell mutants of different sensitivity to chemical carcinogens. Cell mutants previously isolated in this section from a BALB/3T3 clone were classified into four groups according to their different sensitivities to the chemical carcinogens. Preliminary results suggest that there is no major difference in the ability to metabolize benzo(a)pyrene (BP) and the binding of BP to cellular DNA between these mutants. Only one type of BP-nucleoside adduct was found when the cultured BALB/3T3 clones were exposed to BP, whereas it has been reported that many types of adducts were found when BP-metabolites were reacted with isolated DNA in vitro.

In order to search for an active molecule primarily responsible for cell malignancy, we examined whether there is a complementation group between the heat sensitive (hs) and cold sensitive (cs) cell mutants affected in the expression of transformed phenotype which we previously isolated and characterized. Many cell hybrids between hs-mutants, cs-mutants and wild type have been isolated and their properties characterized. It was found that the isolated cell hybrids show various temperature-dependencies for the expression of the transformed phenotype. These results combined with the preliminary karyotype analysis suggest complementation exists between the mutants.

The last major area of investigation concerns photo-chemical carcinogenesis. A combination of psoralen and long wavelength ultraviolet light (UV-A) is being used experimentally and clincally in humans for treatment. This combination has been shown to cause mutations in bacteria and skin cancer in mice. Based on our previous findings of significant reduction in DNA synthesis in circulating leucocytes from some patients with psoriasis immediately after treatment with 8 methoxypsoralen (8MOP) plus UV-A, we attempted to develop an in vitro model to measure the effects of 8MOP plus UV-A on human cells. The results obtained suggest that the low doses of UV-A and 8MOP received by the patients' lymphocytes during therapy may be sufficient to explain the decreased DNA synthesis found in their circulating leucocytes. A surprising observation was that the ultraviolet light source in the absence of psoralens causes a significant decrease in DNA synthesis and inhibition of cell growth of human lymphoblastoid cells. We are attempting to determine the portions of the spectrum causing this effect and the contribution of DNA repair to this long-wave length ultraviolet sensitivity.

Molecular Carcinogenesis Section - *"Studies (1) the metabolic effects on tissues and cells resulting from their exposure of chemical carcinogens and the relationship between these effects and malignant transformation; (2) the metabolic activation and detoxification of the polycyclic hydrocarbons and the relationship of this metabolism of individual susceptibility."* This section studies the molecular events of malignant transformation induced by chemical carcinogens, in particular those of the polycyclic hydrocarbon class. The aim is to understand the enzymatic conversion of carcinogens to either detoxification forms or to the active carcinogenic metabolite. During evolution humans have been exposed to foreign chemical compounds (including carcinogens) and has developed metabolic systems for their detoxification

and elimination. These systems primarily involve microsomal cytochrome P-450 mixed-function oxygenases, but also include epoxide hydratases and conjugating enzymes. The vast majority of foreign compounds are processed by these enzyme systems. The mixed-function oxygenases are influenced by a variety of environmental factors such as drugs, pesticides or carcinogens, and are influenced by the nutritional and hormonal state of the animal. The age, sex and genetic makeup also determines enzyme activity. Work in this laboratory provided the key studies which showed this enzyme system to be responsible for the activation of procarcinogens to what may be their ultimate carcinogenic form.

A primary goal is to define the enzymatic mechanism by which polycyclic hydrocarbons are either activated to carcinogenic forms or to detoxified products. As these enzymes are characterized and sensitive methods are developed for their assay, it may be possible to characterize an individual's enzymatic makeup with respect to carcinogen metabolism and to understand the relationship between this metabolism and individual susceptibility to polycyclic hydrocarbon carcinogenesis. The known sequence of PCH metabolism is as follows: The first step is oxygenation by the microsomal mixed-function oxygenases, one indicator of which is aryl hydrocarbon hydroxylase (AHH). Very sensitive methods have been developed for the latter (see below) which are applicable to human tissues. Secondly, epoxides are hydrated to dihydrodiols by oxide hydratase. The oxides are also metabolized by glutathione S-transferases. The phenols and dihydrodiols are conjugated by UDP glucuronic acid. Sensitive assays (see below) for each of these systems as well as a method for total metabolite analyses by high-pressure liquid chromatography (HPLC) have been developed.

Highly purified cytochrome P-450 LM_2 and LM_4 and partially purified cytochrome P-450 LM_1 and LM_7 exhibit different catalytic activities in the metabolism of benzo(a)pyrene (BP) and (-)trans-7,8-diol. The different cyt-P-450's also exhibit differences in the activation of BP and (-)trans-7,8-diol to intermediates that bind to DNA. Our latest studies showed that in addition to the known ethyl acetate extractable metabolites (tetrols and triols of BP) large amounts of non ethyl acetate extractable metabolites are also formed. Some of these metabolites are bound to proteins. Both types of metabolites were analyzed by high pressure liquid chromatography and paired ion HPLC. The covalent binding to the individual P-450 LM's proteins by metabolites of (-)trans-7,8-diol parallels both (-)trans-7,8-diol metabolism and *in vitro* DNA binding catalyzed by the individual P-450 LM's. LM_4 was more active than LM_2 and LM_1 in binding of the metabolites. No detectable binding was observed with P-450 LM_7 even though it had relatively high metabolic activity.

In another study we showed further evidence for the high degree of stereoselectivity in the activities of cyt-P-450's in the conversion of (-), (+), and (±) trans-7,8-diol to the carcinogenic and mutagenic diol-epoxides I and II. LM_4 has a preference to insert the oxygen below the plane of the double bond of trans-7,8-diol, thus more diol epoxide I is formed from (-) enantiomer and more diol epoxide II from the (+) enantiomer. LM_2 has equal preference to insert the oxygen for the both sides of the trans-7,8-diols.

Thus our studies indicate the large heterogeneity of the cytochrome P-450's in metabolism and activation to forms binding to DNA and protein. This may lead to better understanding of the metabolic pathways of polycyclic hydrocarbon activation and detoxification.

Blood monocytes and lymphocytes from humans were used to assess in vitro an individual's ability to metabolize polycyclic aromatic hydrocarbons to active carcinogens or mutagens. Major improvements have been made in procedures used for the analysis of BP metabolism by these cells which have low levels of mixed-function oxidases. The sensitivity of detection of BP metabolism by HPLC was increased by first separating unreacted BP from the BP metabolites by silica gel column chromatography. Autooxidation of BP metabolites during handling and storage of samples was minimized by the continuous presence of the antioxidant, Vitamin E.

The HPLC analysis of BP metabolism by monocytes and lymphocytes yield the following conclusions: (1) Freezing and thawing of lymphocytes results in little or no quantitative or qualitative difference in their in vitro BP metabolism. In the case of monocytes, freezing and thawing quantitatively decreases in vitro BP metabolism, but there is no qualitative difference. (2) BA-induced monocytes and lymphocytes have a pattern of BP metabolites which is quantitatively greater but qualitatively similar to that of their noninduced counterparts. (3) Comparison of BP metabolism by BA-induced monocytes and lymphocytes which are isolated from the same person reveal quantitative differences in the ratios of BP-phenols and BP-diols. (4) There are quantitative differences in the ratios of BP metabolites when either monocytes or lymphocytes from different individuals are compared.

The metabolism of BP-7,8-diol by monocytes and lymphocytes was analyzed by HPLC. Tetrol I-1 was the predominent product of both monocytes and lymphocytes whether BA-induced or noninduced. In both monocytes and lymphocytes the amount of aqueous-soluble metabolites was substantial, ranging from 50% to 80% of the total 7,8-diol metabolites. When compared to either total organic and aqueous soluble 7,8-diol metabolites or to only organic-soluble 7,8-diol metabolites, 7,8-diol metabolism was not induced by BA to the same extent as was the in vitro BP metabolism. These studies seek to clarify the relationship between individual differences in BP metabolism and carcinogen susceptibility.

(-)r,7,t-8-dihydroxy-7,8-dihydrobenzo(a)pyrene (7,8-dihydrodiol) is one of the metabolic products obtained when benzo(a)pyrene is incubated with rat liver microsomes, this metabolite is further activated to the highly reactive diol epoxide I and II that have been administered to bind to DNA and to be the most powerful mutagen of all the known benzo(a)pyrene metabolites. In view of the potential importance of DNA binding in the initiation of the carcinogenic process we have developed an assay by using ^{3}H(-)7,8-dihydrodiol to study the microsomal catalyzed covalent binding of this substrate to DNA. With this method we have studied the effect on DNA binding of butylated hydroxytoluene and L-ascorbic acid that have been found as protective agents against liver damage, aging and chemical carcinogenesis by using microsomes from control, phenobarbital and 3-methylcholanthrene treated rats. We also studied the effect of 7,8-benzoflavone, glutathione and albumin on the binding to DNA of the (-)7,8-dihydrodiol.

Microsomes from phenobarbital and 3-methylcholanthrene treated rats were 3 and 16 fold more active than control microsomes. DNA binding was inhibited by 7,8-benzoflavone and butylated hydroxytoluene. The extent of inhibition ranged up to 80% and depended on the inhibitor and the type of microsomes. L-ascorbic acid had no effect on DNA binding. DNA binding was also inhibited by 41% in the presence of glutathione and glutathione transferase, and stimulated 52% in the presence of glutathione alone. An increase in the binding (65%) was also observed when albumin was added to the reaction mixture.

The binding of benzo(a)pyrene-4,5-oxide to DNA was increased two fold by albumin and inhibited about 50% by glutathione in the presence or absence of transferase. The increase in (-)r-7,t-8-dihydroxy-7,8-dihydrobenzo(a)-pyrene DNA binding caused by albumin, is likely related to an increase in the half life of its reactive metabolic intermediates, diol epoxides I and II, as well as to an increased metabolism of the parent substrate in the presence of the albumin. The results with butylated hydroxy-toluene may relate to its inhibitory effect on polycyclic aromatic hydrocarbon carcinogenesis.

Nucleic Acids Section - *"Studies (1) interaction of chemical carcinogens and their analogs with nucleic acids and with enzymes regulating nucleic acid metabolism; (2) the effect of these agents on the subcellular organization of nucleic acid macromolecules."* The primary areas of research in this section are: (1) Repair of DNA associated with the biological consequences caused by physical and chemical agents. (2) The characteristics of codon recognition of isoacceptor aa-tRNAs in different species, tissues and neoplasms. (3) Modification of DNA by chemical carcinogens and mutagens. (4) Effects of heavy metals on the biological macromolecules and their muta-carcinogenic consequences.

By the use of the benzoylated naphthoylated DEAE cellulose method, DNA repair synthesis was measured in cell lines derived from normal individuals and patients with the autosomal recessive disease Ataxia teleangiectasis (AT) after irradiation with ultra violet light (UV), methylmethanesulfonate (MMS) and N-methyl-N'-nitro-N-nitrosoguanidine, (MNNG). All AT strains tested had normal levels of DNA repair synthesis after treatment with MMS or UV. UV induced six times more repair synthesis than did MMS.

Relative intracellular thymidine pools were measured to allow for accurate calculation of specific repair activity. Four AT cell strains exhibited only 21-50% of DNA repair synthesis of normal human cell strains after MNNG treatment. Two AT strains showed normal levels of MNNG-induced repair. These findings are significant since previous reports by others showed that AT repair responded only to X-rays. Semiconservative DNA synthesis immediately after MNNG treatment was diminished equally in all normal and AT strains tested.

The previously developed adenovirus host-cell plaque assay (in which adenovirus 2 or 5 is treated with physical or chemical agents and is then assayed for biological activity on monolayers of human cells) was used in three studies: 1) Fibroblasts from persons afflicted with Cockayne's syndrome were found less able than fibroblasts from apparently normal persons to repair DNA damage due to UV; 2) UV was found to cause mutations (reversions) in an adenovirus 5 temperature sensitive mutant and; 3) MNNG treatment of human kidney carcinoma cells was found to decrease their capacity to repair MNNG treated adenovirus 5. This is the first evidence that carcinogens may enhance damage by altering the repair mechanisms.

Aminoacyl-tRNAs from human and rabbit reticulocytes have been compared. Chromatography of all 200 aa-tRNAs from both tissues have shown similar isoacceptor patterns in most cases. It is interesting to note that similar codons are used in human and rabbit globin in RNAs (Kafatos et al., Proc. Natl. Acad. Sci., 74: 5618, 1977).

Patterns of codon recognition of isoacceptor aa-tRNAs from Hymenoptera and wheat germ have been examined. Patterns of codon recognition in Hymenoptera are similar to those previously observed in mammalian tissues. However, several isoacceptor aa-tRNAs are more abundant in Hymenoptera than in mammals, e.g. Ala-$tRNA_{GCG}$. Patterns and abundances of several isoacceptors in wheat germ are different than those in Hymenoptera and mammals,

Phe-tRNA from neoplastic tissues which lacks the characteristic wye base, and Phe-tRNA from normal tissues which contains the wye base, have been compared. Codon recognition properties of both Phe-tRNAs were indistinguishable with regard to wobble in the 5', 3' or middle positions.

It has been found that the binding of a metabolically activated form of benzo(a)pyrene trans-7,8-diol-9,10-epoxide, to adenine bases resulted in severe structural modification of DNA, while binding to guanine bases although quantitatively greater did not cause any significant steric hindrance of the super helical circular DNA of SV 40 virus and plasmid Col El. The presence of some heavy metals ions such as Pb, La, Ni and Se in the reaction mixture enhanced the binding (to DNA bases as well as phosphate groups) and the modification of DNA by diol-epoxide B(a)P. These metal ions were found to have little, if any, mutagenic activity in Salmonella typhimurium. However, they markedly promoted the mutagenic activity of a minimum dose of diol-epoxide which was added to the mutagenesis assay system. The co-mutagenic and co-carcinogenic effects of heavy metal ions which are often present together with polycyclic hydrocarbons in the environment are, therefore, considered to be of particular importance.

The mechanisms of induction of a low molecular weight protein (metallothionein) which specifically binds to heavy metal ions in the cell was also studied extensively using a newly established tissue culture system. The protein thus induced might play a preventive role against the toxic and co-mutagenic activities of heavy metals.

Protein Section - *"Studies (1) the relationship of proteins to the carcinogenic process; (2) interactions between carcinogenic agents and proteins; (3) changes in proteins occurring as a result of or concomitant with the administration of carcinogenic agents; relates the genetic control of such proteins to inheritance in laboratory animals and man."* Significant progress has been made in the preparation of heterogeneous nuclear RNA from HeLa cells, resulting in preparations which give reproducible results in electrophoresis. Because the resolution afforded by the electrophoresis is substantially superior to that obtained with sucrose gradient analysis, it becomes increasingly urgent to establish the absolute lengths (in nucleotides) of these molecules and to correlate size measurements made by the two techniques. We are in the process of developing a novel method for estimating nucleotide lengths which is independent of the polynucleotide's hydrodynamic or electrophoretic properties. Briefly, the method depends upon a comparison between the population of molecules present after thermal degradation with the population expected on the basis of well-established theory. Although the technique is still under development, sizes determined by the new method agree closely with those established by other methods for certain standard RNAs. Our results at present indicate that the heterogeneous nuclear RNA is substantially more homogeneous and significantly smaller than previously believed. A previously described apparatus has been modified for elution of RNA from preparative slab gels. RNA was recovered from the gel with modest yields, but with almost no degradation.

Studies on factors controlling the synthesis of haptoglobin have been continued using freshly isolated hepatocytes from rat liver in an in vitro system. In order to evaluate the synthesis rate of haptoglobin and albumin (used as a control) solid-phase affinity reagents were prepared using hemoglobin and antibody to rat albumin, respectively. Studies with this system showed that the addition of cortisol to the medium caused an increase in the rate of synthesis of haptoglobin relative to albumin. Additions of either intact haptoglobin or haptoglobin from which either the terminal sialic acid or the sialic acid and adjacent galactose had been removed failed to alter the synthesis rates, although both modified haptoglobins were bound to the membranes of the hepatocytes. The results deny the hypothesis under study and indicate that the binding of the modified haptoglobin does not constitute the signal for its synthesis. Bacterial lipopolysaccharide and prostaglandins E_1 and E_2 also failed to alter the relative synthesis of haptoglobin and albumin. Other substances such as complement components and pyrogene factors from stimulated leukocytes which may serve as signals are under study.

Earlier work on the identification of phosphoproteins and sialoglycoproteins in rodent mammary tissue has been extended to surgical specimens from normal and malignant human mammary tissue. As was the case earlier, the simple staining method gives information concerning the types of macromolecules present in connective tissue and in secretions within cells, alveolar lumina and ducts. In a bioassay for prolactin developed earlier, based on the production of casein, it was noted that the accumulation of a protein near the 93,000 molecular weight marker on SDS gels was also proprotional to the prolactin concentration of the medium. Further studies indicate that this protein is lactoferrin. Studies on the potential mutagenic activity of

estrone and related steroids have continued. Detailed statistical analysis of earlier data has confirmed a statistically significant increase in the number of colonies formed in the Ames test when some of these compounds and S-9 are incubated on the plates. Because the frequency of mutation is low, efforts to find optimum testing conditions for this class of compounds are underway.

Experiments to identify microsomal proteins (cytochromes P-450) by electrophoretic methods were continued. The same technique which had been useful in showing clear differences in the composition of liver microsomal proteins from carcinogen-treated and untreated rats were applied to rat liver cells growing in tissue culture and to preparations from human monocytes and lymphocytes. In some cases the microsomal proteins were labeled with radioactive amino acids. It was not possible to observe consistent changes following enzyme induction nor even to identify molecules comparable to those observed in liver. Modifications in the electrophoretic techniques are being explored in the hope of improving the analysis for these important enzymes. In addition, spectrophotometric examination of liver microsomes and whole and subfractionated cells will be made to see if differences in these hemoproteins can be detected using this method.

Studies centered around chromatin proteins and structure have been initiated this year. The major aim of one project involves the repair of UV-induced damage to DNA. The location of the repair site is being studied with respect to the nucleosome structure; that is, whether repair occurs in the portion of the DNA tightly coiled about the nucleosome or in the bridge regions. In addition, non-histone proteins which bind lectins are under investigation as a portion of a larger project in which immunochemical techniques are used to study the structure of various functional states of chromatin and chromosomes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04474-02 CH

PERIOD COVERED
October 1, 1977 to June 30, 1978

TITLE OF PROJECT (80 characters or less)

Factors Controlling Hepatic Synthesis of Acute-Phase Plasma Glycoproteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. C. Hooper	Research Associate	CH NCI
OTHER:	A. C. Peacock	Head, Protein Section	CH NCI
	C. J. Steer	Clinical Associate	DD NIAMDD

COOPERATING UNITS (if any)

Digestive Diseases Branch, NIAMDD

LAB/BRANCH
Chemistry Branch, Carcinogenesis Research Program

SECTION
Protein Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	1.2	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Potential mediators of the acute-phase plasma glycoprotein response to inflammation were studied in primary suspension cultures of rat hepatocytes in vitro. ^{3}H-Leucine incorporation into haptoglobin was compared with that of albumin as a measure of the synthesis of an acute-phase glycoprotein relative to that of a protein not participating in the acute-phase plasma protein response. The addition of hydrocortisone or a hormone mixture containing hydrocortisone, glucagon, triiodothyronine, and somatotropin to the hepatocyte suspensions produced a differential stimulation of haptoglobin synthesis. Among factors demonstrating no effect on the relative synthesis of haptoglobin in vitro are insulin, endotoxin, prostaglandins E_1, E_2, A_1, A_2 and $F_{2\alpha}$ and asialo- and asialo-, aglacto- preparations of haptoglobin and orosomucoid. Currently under investigation as other potential mediators are complement components and pyrogenic products from stimulated leukocytes.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04474-02-CH

Project Description

Objectives: To determine *in vitro* the factors that control the increase in plasma acute-phase glycoprotein synthesis by hepatocytes in response to inflammation *in vivo*.

Methods Employed: 1) Primary hepatocyte suspension culture, 2) solid-phase hemoglobin-binding radioassay for haptoglobin, 3) solid-phase radioimmunoassay, 4) gel filtration and ion exchange chromatography, and 5) polyacrylamide gel electrophoresis.

Major Findings: Rat hepatocytes isolated with high viability by collagenase perfusion were shown to incorporate ^{3}H-leucine into haptoglobin approximately linearly for up to 48 hours in primary suspension culture. ^{3}H-Leucine incorporation into albumin was linear for 24 hours, but slowed from 24 to 48 hours incubation. No greater than 10-30% degradation of these proteins occurred under the standard culture conditions, and over 90% of the radioactivity incorporated into haptoglobin and albumin was secreted into the medium throughout the 48-hour incubation and could be inhibited by 3 μM cycloheximide.

Consistent stimulation of ^{3}H-leucine incorporation into haptoglobin relative to that into albumin was found with the addition to the insulin-supplemented culture medium of hydrocortisone or a hormone mixture containing hydrocortisone, glucagon, triiodothyronine, and somatotropin. Asialo- and asialo-, agalacto- preparations of haptoglobin and orosomucoid were shown to bind to the isolated hepatocytes, but were without demonstrable effect on leucine incorporation into either haptoglobin or albumin whether in the presence or absence of glucocorticoids. Bacterial lipopolysaccharide and prostaglandins E_1, E_2, A_1, A_2, and $F_{2\alpha}$ also failed to stimulate haptoglobin synthesis differentially *in vitro*.

Significance to Biomedical Research and the Program of the Institute: A major aspect of the systemic response to inflammation is a striking increase in the hepatic synthesis of several plasma glycoproteins with little change in albumin synthesis. Although glucocorticoids have been shown to have a permissive effect *in vivo*, and crude preparations of stimulated leukocytes have accelerated synthesis of plasma glycoproteins *in vivo* and *in vitro*, the specific factors mediating this response are uncertain. The development of an *in vitro* system utilizing freshly isolated hepatocytes and a solid-phase hemoglobin radioassay has permitted confirmation of the involvement of glucocorticoids in regulation of the synthesis of haptoglobin, a representative plasma acute-phase glycoprotein. Other factors previously shown to be involved in other aspects of inflammatory responses *in vivo*, endotoxin (circulatory collapse, fever) and prostaglandin E_1 (increased local vascular permeability) were shown not to affect hepatocyte synthesis of haptoglobin *in vitro*. This work also represents the first direct demonstration *in vitro* that the binding of the partially deglycosylated plasma glycoproteins (asialo- and asialo-, agalacto-haptoglobin and orosomucoid) to their hepatocyte plasma membrane receptors is without effect on the subsequent synthesis of the same or other

acute-phase glycoproteins. This finding is of particular interest in the context of an earlier hypothesis that the previously elucidated binding of asialo-glycoproteins to hepatocyte membranes constituted a feedback signal for glycoprotein synthesis, which was augmented in inflammatory states by a local release of leukocyte lysosomal hydrolases (including neuraminidase).

The phenomena under investigation are common to inflammatory states induced by infectious diseases and cancer. Elevated levels of plasma glycoproteins are of uncertain value to the host, although the ability to bind released proteolytic enzymes or free hemoglobin released by hemolysis may be of protective value in infectious diseases, leukemia, and other neoplastic conditions. Study of cell surface receptors and recognition phenomena, such as those involving sialic acid and other carbohydrate moieties, are also important in understanding regulation of cell growth and replication.

Proposed Course of Project: Additional potential mediators of the plasma acute-phase glycoprotein response to be studied include complement components and pyrogenic products from stimulated leukocytes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04473-02 CH

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Studies on the Control of Benzo(a)pyrene Metabolism in Cells from Animal and Human Sources

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. Okano	Staff Fellow	CH NCI
OTHER:	H. V. Gelboin	Chief, Chemistry Branch	CH NCI
	H. Miller	Chemist	CH NCI
	R. Robinson	Biologist	CH NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Chemistry Branch, Carcinogenesis Research Program

SECTION
Molecular Carcinogenesis

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.9	1.1	1.8

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
(1) Blood monocytes and lymphocytes, a readily available source of human tissue, are used to assess in vitro an individual's ability to metabolize benzo(a)-pyrene, a model compound of the class of polycyclic aromatic hydrocarbon carcinogens. A mammalian mutagenesis system using Chinese hamster V-79 cells will be used to assess the ability of human blood monocytes and lymphocytes and rat liver and mouse cells to activate polycyclic hydrocarbons to active mutagens.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: (1) To determine whether quantitative and/or qualitative differences exist in the *in vitro* metabolism of benzo(a)pyrene in human peripheral blood monocytes and lymphocytes under varying treatment and incubation conditions. (2) To examine the ability of blood monocytes and lymphocytes and rat liver or mouse cells to activate polycyclic hydrocarbons to active mutagens in the Chinese hamster V-79 mutagenesis system.

Methods Employed: Lymphocytes and monocytes are separated from other components of fresh human blood by density gradient centrifugation on Ficoll-Hypaque. Separation of monocytes from lymphocytes is by the differential attachment of monocytes to plastic cell culture dishes. Cells are cultured and treated according to previously published procedures. (2) Rat liver and mouse cell cultures were obtained from sources at NIH. (3) After incubation of cells in the presence of benzo(a)pyrene (BP), the relative AHH activity and inducibility of cells is determined by a fluorescence assay using 3-hydroxy-BP as a standard. (4) Cells are also incubated in the presence of radiolabeled BP and BP-(-)*trans*-7,8-dihydrodiol. Metabolites extracted from the reaction mixture are analyzed by high pressure liquid chromatography (HPLC). (5) Mutagenesis in Chinese hamster V-79 cells will be assayed according to the previously published procedures of others.

Major Findings: (1) The relative activity and inducibility of the mixed-function oxidases in cultured monocytes and lymphocytes from various individuals have been compared by the analysis of BP metabolism by HPLC. Both monocytes and lymphocytes can metabolize BP to products which include: 9,10-, 4,5- and 7,8-dihydrodiol, 9-, 3- and 7-phenol, 1,6- and 3,6-quinone and 2 unknowns. The data suggests that BA-induced monocytes and lymphocytes and BA-induced and control cells differ quantitatively but are qualitatively similar in their ability to metabolize BP. Both monocytes and lymphocytes are capable of metabolizing BP-7,8-diol further to the highly mutagenic and carcinogenic BP-7,8-diol-9,10-epoxide. Preliminary experiments suggest that varying divalent cation concentration quantitatively affects BP metabolism in monocytes and lymphocytes. (2) The pattern of BP metabolism has been determined for two rat liver cell lines and for mouse 3T3 cells. In one rat liver cell line little or no detectable dihydrodiols are formed.

Significance to Biomedical Research and the Program of the Institute: Peripheral blood mononuclear leukocytes, a readily available source of human tissue, have the ability to metabolize polycyclic aromatic hydrocarbon carcinogens. These cells use the same microsomal mixed-function oxygenase system, aryl hydrocarbon hydroxylase, which is found in other tissues. Analysis of Benzo(a)-pyrene metabolism by the AHH assay and by HPLC will yield quantitative and qualitative data which will hopefully be correlated to carcinogenic potential. The eventual goal is the development of *in vitro* systems which can assess an individual's relative susceptibility to polycyclic aromatic hydrocarbon carcinogenesis.

Proposed Course of the Project: Blood monocytes and lymphocytes from various individuals who have different relative AHH activities and inducibilities will continue to be assayed for BP metabolism by HPLC. Blood monocytes and lymphocytes and rat liver and mouse cells will be used to activate polycyclic hydrocarbons to mutagens in the Chinese hamster V-79 mutagenesis system.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04496-01 CH

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

In situ Organization of Chromosomal Components

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: M. Bustin Visiting Scientist CH NCI

COOPERATING UNITS (if any)
Laboratory of Chemical Immunology, Weizmann Institute of Science, Israel
Laboratory of Chemical Physics, Weizmann Institute of Science, Israel
Department of Biology, Johns Hopkins University, Baltimore, MD

LAB/BRANCH
Chemistry Branch, Carcinogenesis Research Program

SECTION
Protein Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 1.5	PROFESSIONAL: 0.75	OTHER: 0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Probes, specific for defined chromosomal components, facilitate the study of the structure of various functional states of chromatin and chromosomes. Antibodies specific for individual histone fractions are used to study the organization of these proteins, in metaphase and polytene chromosomes, as well as in the transcribed and nontranscribed regions of the genome. Immunochemical techniques are used to study the composition and heterogeneity of purified nucleosomes. Immunofluorescence and immunoelectron microscopy allow visualization of the location of particular histones in chromosomes, in metabolically silent chromatin, and in chromatin actively undergoing DNA replication and RNA transcription. Antibodies against purified non-histone proteins (HMG) have been elicited. These antibodies bind to chromatin and can be used to study the structure-function relationship of these proteins.

PHS-6040
(Rev. 10-76)

Cooperating Units (continued)
Department of Biology, University of Virginia, Charlottesville, VA
Department of Biochemistry, University of Oregon, Corvallis, OR

Project Description

Objectives: To understand the role of defined chromosomal proteins in maintaining the structure and regulating the function of chromatin and chromosomes. To explore the possibility that neoplastic transformation is associated with defined alterations in either the type or the organization of chromosomal components.

Methods Employed: Histones are extracted from calf thymus and purified by gel and affinity chromatography. The histones are complexed with RNA, and injected into rabbits at multiple intradermal sites. Antisera are tested by microcomplement fixation. The IgG fraction from these sera is obtained by chromatography on DEAE cellulose. To obtain purified antibodies, histones are covalently bound to Sepharose by the cyanogen bromide procedure and either sera or the IgG fraction passed on these columns. The activity of these antibodies is checked by the microcomplement fixation technique. The purity of the preparation is monitored by immunodiffusion and by immunoelectrophoresis.

Non-histone proteins of the HMG class are extracted from purified calf thymus chromatin by low salt, and purified by ion exchange chromatography and electrophoresis on polyacrylamide gels. Antibodies are elicited in rabbits. The procedure for purification of these antibodies is very similar to that described for histones. The activity of the antibodies can also be tested by precipitin.

Proteins are iodinated by either the chloramine T or by the peroxidase technique. Chromatin is obtained from isolated nuclei and purified by centrifugation through 1.7 M sucrose.

Cells are obtained from various tissues by gentle disruption with a Dounce homogenizer. EBTr (Embryonic bovine trachea) cells obtained from the American Type Culture Collection are grown in culture flasks to confluency. The confluent cells are dislodged by trypsin treatment, seeded on either microscope slides or cover slips and allowed to attach by overnight incubation.

Chromatin subunits (nucleosomes) are obtained from isolated nuclei or purified chromatin by digestion with micrococcal nuclease.

Metaphase chromosomes are obtained by metaphase arrest of cultured cells using colchicine. Polytene chromosomes are obtained from salivary glands harvested at the 4th instar stage of a larvae of a laboratory strain of *Chironomus thummi*. Squashes of such cells were fixed with formaldehyde.

Metabolically active chromatin was obtained from single pre-staged *Drosophila* embryos. The embryos were disrupted in sodium borate, pH 8.5, and centrifuged through a 0.1 M sucrose, 10% formalin solution onto a carbon-coated electron

microscope grid. The grid was treated with either antibody to histones, purified by affinity chromatography, followed by ferritin-labelled goat anti-rabbit gamma globulins, or directly with ferritin-labelled $F(ab)_2$ portion of the purified anti-histone antibodies. The location of histone antigenic determinants in either metaphase chromosomes or polytene chromosomes from Chironomus thummi was visualized by the indirect immunofluorescence technique.

Major Findings: Purified histone fractions elicit specific antibodies. The antibodies belong to the IgG class. Their behavior on DEAE cellulose is anomalous in that they elute at a higher elution volume than most IgG fractions obtained from rabbits. This anomalous chromatographic behavior probably reflects the inverse relationship between the positive charge of the antigen and the resulting relatively negatively charged antibody.

Antibodies elicited by histones bind specifically to isolated chromatin, purified nucleosomes, metaphase chromosomes and polytene chromosomes. Only part of the antigenic sites present in histones are exposed and available to interact with antibodies. The number of antigenic sites exposed in chromatin-bound histones varies among the various histone fractions. By measuring the ability of chromatin purified from various sources to interact with specific histones it is possible to detect differences among chromatins and possibly follow structural changes associated with various functional states of the cell.

Each chromosome present in metaphase-arrested cells contains each histone fraction evenly distributed along the entire length of the chromosome. In polytene chromosomes, however, it is possible to obtain distinct banding patterns by treatment with anti-histone antibodies and examination by the indirect immunofluorescence technique. The fluorescence is more intense in the bands than in the interbands, suggesting that the amount of fluorescence is correlated with the amount of DNA in a band. Antisera against each of the purified histone fractions gave similar fluorescence, suggesting that the gross organization of the various histones is similar in the various regions of the chromosome. The fact that the fluorescence is proportional to the amount of DNA suggests that the accessibility of the antigenic determinants in chromatin-bound histones is similar in the band and interband regions of the chromosome.

By treatment with various salt concentrations or with ectdysone it is possible to induce puffing at specific loci in the chromosome. This puffing is analogous to specific gene induction. A marked decrease in the intensity of fluorescence of the puffed regions of the polytene chromosomes is observed. Apparently the structure of the active chromatin fiber is such that histones are preferentially lost from these regions. Thus it is possible to use the anti-histone sera as specific cytological markers to detect structural changes associated with defined functional changes occurring in particular regions of the genome.

At a higher level of resolution immunoelectron microscopic techniques are used to study the organization of histones in metabolically active chromatin. Using pre-staged Drosophila embryos it could be shown that both the non-ribosomal transcribed regions of the genome as well as the replicating regions

of the genome contain histones. Thus, the fundamental packing of chromatin in the metabolically active regions of the genome is similar to the structure of the interphase chromatin regions.

Using immunoelectron microscopic techniques it was possible to visualize the location of an antibody molecule at the periphery of the nucleosome. Thus, in future studies, it may be possible to define the domain which a particular histone occupies within the nucleosome. Furthermore, each nucleosome in a tissue will bind anti-H_2B antibodies, suggesting that each nucleosome contains this histone. By binding purified antibodies to isolated nucleosomes and subjecting the reaction mixture to ultracentrifugation it was found that all the nucleosomes displayed an increased molecular weight. This finding was observed with each of the antibodies, suggesting that each nucleosome contains all the histones in equimolar ratios. While the histone composition of the nucleosomes is identical, the exposure of histone antigenic determinants in the nucleosomes displays some heterogeneity since with anti-H_3, anti-H_4 and anti-H_2A sera not all the nucleosomes bound antibodies. Possibly nucleosomes differ in their content of non-histone proteins and this difference accounts for the immunological difference observed when nucleosomes are reacted with anti-histone antibodies and examined by electron microscopy.

In an effort to study the organization of non-histone proteins, the non-histone protein HMG-1 from calf thymus has been purified and antibodies against this protein elicited. Using these antibodies the sequence differences between several other related pure non-histone proteins have been approximated. Furthermore, it was found that these antibodies bind to chromatin. Thus their organization in chromatin can be studied and visualized by various immunochemical techniques.

Significance to Biomedical Research and the Program of the Institute: Understanding the mechanism of gene regulation and its relation to neoplasia requires knowledge of the structure of chromatin and chromosomes. The approach developed in this laboratory is presently the only approach in which specific probes for well-defined purified chromosomal components are used to study the organization of these components in intact chromatin and chromosomes. As such, a unique opportunity has developed whereby certain structural aspects of these nucleoproteins can be visualized and directly related to functional stages of the genome. The immunological techniques developed for the study of the *in situ* organization of histones in chromatin and chromosomes are applicable to the study of any chromosomal component which can be purified and against which specific antibodies can be elicited.

Proposed Course of Project: Studies on the organization of histones in metabolically active chromatin by various immunological techniques will be continued. Structural changes in the fundamental structure of the chromatin fiber occurring during transcription and replication will be followed. A radioimmunoassay for nucleosomes will be developed. This assay will be used to screen chromatin and nucleosomes obtained from various types of cells. Immunological techniques will be used to study structural changes in polytene chromosomes associated with gene activation. The location of HMG non-histone

proteins in chromatin and chromosomes will be visualized. The DNA complexity of regions associated with these proteins will be determined.

Publications

Bustin, M.: Histone antibody and chromatin structure. In Sparkes, R. S., Comings, D. E., and Fox, C. F. (Eds.): Human Molecular Cytogenetics, Winter Symposia, New York, Academic Press, 1977, pp. 25-40.

Bustin, M., Reeder, R. H., and McKnight, S. L.: Immunological cross reaction between calf and Drosophila histones. J. Biol. Chem. 252: 3099-3101, 1977.

Bustin, M., Kurth, P. D., Moudranakis, E. M., Goldblatt, D., Sperling, R., and Rizzo, W.: Immunological probes for chromatin structure. Cold Spring Harbor Symposia (in press).

McKnight, S. L., Bustin, M., and Miller, D. L.: Ultrastructural and antigenic properties of metabolically active chromatin. Cold Spring Harbor Symposia (in press).

Bustin, M., Simpson, R. T., Sperling, R., and Goldblatt, D.: Molecular homogeneity of the histone content of HeLa chromatin subunits. Biochemistry 16: 5381-5385, 1977.

Bustin, M.: Histone antibodies: Structural probes for chromatin and chromosomes. In Busch, H. (Ed.): The Cell Nucleus, New York, Academic Press (in press).

Goldblatt, D., Sperling, R., and Bustin, M.: Heterogeneity in the interaction of chromatin subunits with anti-histone sera visualized by immunoelectron microscopy. Exp. Cell Res. 121: 1-14, 1978.

Bustin, M., Hopkins, R. B., and Isenberg, I.: Immunological relatedness of HMG chromosomal proteins from calf thymus. J. Biol. Chem. 253: 1694-1699, 1978.

Bustin, M.: Binding of E. coli RNA polymerase to chromatin subunits. Nucleic Acids Res. 5: 925-932, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04497-01 CH

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Glycoproteins in Chromatin and Chromosomes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: M. Bustin Visiting Scientist CH NCI

COOPERATING UNITS (if any)

Department of Biology, Johns Hopkins University, Baltimore, MD

LAB/BRANCH
Chemistry Branch, Carcinogenesis Research Program

SECTION
Protein Section

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.45	0.20	0.25

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Concanavalin A binds specifically to chromatin. Electrophoresis in polyacrylamide gels in the presence of SDS reveals that 80% of the concanavalin A binding capacity is localized in one non-histone protein with an apparent molecular weight of 135,000. Fluorescence and autoradiographic studies reveal that this lectin binds to specific loci in polytene chromosomes. The binding site may change during the life cycle of a cell. Attempts are being made to isolate the con-A receptor. Glycoproteins may have important functions for various recognition phenomena important for proper functioning of chromosomes and chromatin.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04497-01-CH

Project Description

Objectives: To detect and purify glycoproteins present in chromatin and chromosomes and determine their function in these nucleoproteins.

Methods Employed: Chromatin is isolated from purified nuclei obtained from various sources. Lectins, simple sugars and complex carbohydrates are commercially purchased. Lectins were labelled with ^{125}I by the lactoperoxidase technique. Proteins are extracted from chromatin by the phenol technique. The molecular weight of lectin-binding components is determined by subjecting the proteins to electrophoresis in polyacrylamide gels run in the presence of SDS. The stained gel is washed and incubated with radioactive lectins. The location of the radioactive lectin, specifically bound to the gel, is visualized by autoradiographic techniques.

Polytene chromosomes are prepared from salivary glands harvested at the 4th instar stage of a larvae from a laboratory strain of Chironomus thummi. The location of lectin-binding components in these chromosomes is visualized by fluorescence microscopy using fluorescein- or rhodamin-labelled lectin or by autoradiography using radioactively labelled lectins.

Major Findings: Concanavalin A binds specifically to chromatin purified from either rat liver or rat thymus. The binding is reversible. It is inhibited only by sugars for which concanavalin A has a binding site, attesting to the fact that the binding does not represent a non-specific trapping of the lectin to chromatin. In the rat liver there is one concanavalin A binding site per 1400 base pairs of DNA. In the rat thymus one binding site per 2800 base pairs of DNA was detected. Scatchard analysis reveals the presence of one type of binding site with an apparent dissociation constant of 3×10^{-7} M. By electrophoresis in polyacrylamide gels three polypeptide bands among the non-histone proteins which specifically bind con-A were identified. Approximately 80% of the iodinated con-A bound to a polypeptide with a molecular weight of 128,000 daltons. The concanavalin A-binding glycoprotein can be extracted from rat liver chromatin by phenol. It is also present in rat thymus chromatin.

In an effort to localize the concanavalin A-binding protein, polytene chromosomes from Chironomus thummi have been reacted with fluorescein-labelled and ^{3}H-labelled concanavalin A and examined by fluorescence microscopy and autoradiography. Specific binding to selected regions of the chromosome was observed. The binding is not confined to either the band or interband regions of the chromosome. Most striking was the finding that a puff in region d of chromosome 4 bound concanavalin A at early stages of puffing. At later stages of development when the puff decreases in size, the binding is significantly reduced. Thus the presence of the glycoprotein can be correlated with the size of a particular puff. To explore the presence of different types of glycoproteins in chromatin the binding of soya bean agglutinin (specific for N-acetyl-D-galactosamine) to rat liver chromatin was examined. Soya bean agglutinin was iodinated with peroxidase. Electrophoresis in polyacrylamide gels run in the presence of sodium dodecyl sulfate verified that ^{125}I was

indeed incorporated into the lectin. No significant binding of soya bean agglutinin to rat liver chromatin was detected, suggesting that the sugar moiety N-acetyl-D-galactosamine is not present in rat liver chromatin.

Significance to Biomedical Research and the Program of the Institute: Various recognition phenomena among nuclear components and between chromosomal and cytoplasmic components may influence processes important for gene regulation. It is known that glycoproteins can function as recognition molecules, especially in the cell membrane. The finding that concanavalin A binds to purified chromatin is the first definite report on the presence of glycoproteins in purified chromatin. These glycoproteins may function as recognition molecules between various regions of the genome, between chromatin and the nuclear membrane, in the scaffolding proteins of the chromosome, or in chromosome pairing during meiosis. Identification and characterization of these components may further our understanding of processes important in gene regulation and in the development and maintenance of the neoplastic state.

Proposed Course of Project: Novel assays for detecting lectin-glycoprotein interactions are being developed. Such assays will facilitate purification of specific glycoproteins. The binding of various lectins to purified chromatin will be explored. Lectin-binding components will be purified and characterized. The location of glycoproteins in the interphase and metaphase chromatin will be studied.

Publications

Rizzo, W. B. and Bustin, M.: Lectins as probes for chromatin structure. J. Biol. Chem. 252: 7062-7067, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04498-01 CH

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Localization of Repaired Sites in Chromatin Following Ultraviolet Damage

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Bustin	Visiting Scientist	CH NCI
	K. Kraemer	Research Scientist	CH NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Chemistry Branch, Carcinogenesis Research Program

SECTION

Protein Section; Cell Growth Regulation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.4	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The mechanism of repair of UV-induced DNA damage is studied. Human lymphoblastoid cell lines, including normal and xeroderma pigmentosum, which differ in their ability to repair UV-induced DNA damage, are exposed to different doses of 254 nm UV radiation. The cells are allowed to repair the damage in the presence of radioactive nucleotides. Chromatin from these cells is isolated and the location of the repair site identified with the aid of specific nucleases. The major aim of the project is to identify repair sites and to explore the possibility that differences among various cells in UV sensitivity reflect some differences in the repair sites.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04498-01-CH

Project Description

Recent studies from other laboratories have suggested that repair of ultraviolet-damaged DNA in chromatin is non-uniform and begins between nucleosomes. The objectives of this project are 1) to try to pinpoint with a greater precision the location of sites of initiation of DNA repair in normal cells, and 2) to determine if cells from patients with cancer-prone genetic diseases which have different defects in DNA repair (such as xeroderma pigmentosum) have abnormal localization of DNA repair sites.

Normal and xeroderma pigmentosum lymphoblastoid cell lines from which large numbers of cells can be obtained rapidly are being studied. Following irradiation from a germicidal lamp the cells are allowed to repair the damage in the presence of tritiated thymidine. Chromatin from these cells is isolated and the location of the radioactive thymidine (representing the repaired sites) is identified by the use of specific nucleases.

The project is still in the early stages of development. It represents a new and different approach to the question of the mechanisms of abnormal DNA repair which are associated with human cancer-prone diseases.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04499-01 CH

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Separation, Identification and Quantitation of Cytochromes P-450 in Mammalian Tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. M. Robie Suh	Staff Fellow	CH NCI
OTHER:	P. R. McIntosh	Visiting Fellow	CH NCI
	A. C. Peacock	Head, Protein Section	CH NCI
	H. V. Gelboin	Chief, Chemistry Branch	CH NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Chemistry Branch, Carcinogenesis Research Program

SECTION
Protein Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Several forms of cytochrome P-450 are known to exist in mammalian liver. The type and amount of these "cytochromes P-450" present are affected by exposure of the animal to some drugs, polycyclic hydrocarbons, and various other substances in the environment. Cytochrome P-450 is the rate-limiting component of a mixed-function oxidase enzyme system which catalyzes the metabolism of many compounds including a large number of those polycyclic hydrocarbons known to produce cancer in humans. The ability of the individual to activate or de-activate chemical carcinogens and thus his susceptibility to cancer induction by a given agent may depend on the absolute amounts or relative proportions of particular cytochrome P-450 species present. Hence, it would be useful to be able to recognize and quantitate the cytochrome P-450 forms. In this study methods for the identification of these proteins are being developed. These methods include 1) SDS-polyacrylamide gel electrophoresis, 2) isoelectric focusing and 3) spectrophotometric examination. The methodology is being developed using rat and rabbit liver. Once established, the methods will be applied to analysis of cytochromes P-450 of human blood monocytes and lymphocytes.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04499-01-CH

Project Description

Objectives: The objectives of this study are: (1) To detect and quantitate multiple cytochrome P-450 forms in various animal and human tissues and to determine the effects of exposure to carcinogenic polycyclic hydrocarbons (e.g., 3-methylcholanthrene and benzo(a)pyrene) on the amounts and proportions of these cytochromes present, and (2) To determine whether there is a relationship between type and/or amount of cytochrome P-450 present in a tissue and the incidence of cancer arising upon exposure to a given carcinogen.

Methods Employed: A combination of analytical polyacrylamide gel electrophoresis under both denaturing and non-denaturing conditions and spectrophotometric methods will be used in this study. Hopefully, a two-dimensional gel electrophoresis method which will allow rapid and precise detection of the various forms of cytochrome P-450 even in the presence of numerous other proteins will be developed. In addition, quantitation of the species of cytochrome P-450 will be done by either (1) spectrophotometric analysis in the presence and absence of appropriate ligands of cellular subfractions or non-denaturing gel eluates containing the cytochrome or (2) spectrophotometric analysis of protein patterns obtained after polyacrylamide gel electrophoresis.

For the development of the analytical method rat and rabbit liver will be used as the source of the cytochrome P-450. Once the reliability of the method has been established, it will be applied to samples of human tissue, including blood monocytes and lymphocytes.

Major Findings: Polyacrylamide gel electrophoresis of rat liver microsomal proteins in the presence of sodium dodecyl sulfate (SDS) reveals about 40 polypeptide bands. Upon treatment of the rat with the carcinogenic polycyclic hydrocarbon 3-methylcholanthrene, the gel electrophoretic pattern is altered. The altered gel pattern is accompanied by a shift in the wavelength of maximal absorbance of the reduced cytochrome P-450-carbon monoxide complex peak from 450 nm to 448 nm as well as by altered levels and substrate specificity of enzyme activity.

On the contrary, Buffalo rat liver cells (mammalian cells in tissue culture) show no changes in the SDS-polyacrylamide gel patterns after polycyclic hydrocarbon treatment even though the levels of enzyme activity are increased. Similarly, no differences were seen in the gel patterns of human lymphocytes or monocytes treated in vitro with polycyclic hydrocarbons although this treatment increased enzyme activity significantly.

Significance to Biomedical Research and the Program of the Institute: This study should clarify the relationship between drug metabolizing ability, cytochrome P-450 type and chemical carcinogenesis, particularly that caused by polycyclic hydrocarbons.

Proposed Course of Project: Most of the remainder of this research period will be devoted to the development of the electrophoretic and spectrophotometric methodology and hopefully will allow sufficient progress to warrant beginning application of the methods to human monocytes and lymphocytes.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-CP-04516-02-CH

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Cellular and Molecular Effects of Psoralen Plus Ultraviolet Light

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: K.H. Kraemer Research Scientist CH NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Chemistry Branch, Carcinogenesis Program

SECTION
Cell Growth Regulation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Photochemical carcinogenesis as typified by the cellular and molecular effects of the combination of psoralen and long wavelength ultraviolet light (UV-A) is being studied. This combination has been found to be mutagenic in bacteria and carcinogenic in mice. It induces DNA-psoralen binding and is being used experimentally for treatment of psoriasis and the cutaneous lymphoma mycosis fungoides. There is a significant decrease in DNA synthesis in circulating leucocytes from some patients following this treatment. The present research involves development of an in vitro assay to measure the effects of psoralen plus UV-A on cell proliferation and DNA synthesis using rapidly dividing human lymphoblastoid cells. These studies may be useful in governing the use of psoralen plus UV-A by indicating conditions of toxicity and possible mutagenicity to human cells and in clarifying the mechanism of cell damage by psoralen.

PHS-6040
(Rev. 10-76)

Project Description

High dose oral psoralen plus high intensity long wavelength ultraviolet light (UV-A) is currently being used experimentally to induce remissions in psoriasis and in the cutaneous lymphoma mycosis fungoides. In the presence of UV-A, psoralen binds to DNA forming mono-adducts and interstrand cross links. This combination has been shown to cause mutations in bacteria and skin cancer in mice. On the other hand, low dose psoralen with low intensity ultraviolet light has been used in humans for many years for treatment of vitiligo with no documented neoplastic sequalae. Thus, the neoplastic potential on humans of this more intense treatment is not known.

Work begun at the University of Miami and continued at NIH as part of this project has demonstrated a significant reduction in DNA synthesis in circulating leucocytes from some patients with psoriasis after treatment with psoralen plus UV-A. The immediate objective of this project is the development of an *in vitro* assay to measure the effects of psoralen plus UV-A on human leucocytes. Long term human lymphoblastoid cell lines were chosen as a model because they were derived from leucocytes, can be rapidly grown to high density in suspension culture, can initiate cultures from small numbers of cells, and have an apparently infinite life span. The effect of psoralen plus UV-A on lymphoblastoid cells is measured in terms of changes in the rate of DNA synthesis (estimated by acid insoluble tritiated thymidine incorporation into cells harvested on Millipore filters and by autoradiography), alterations in the rate of population growth in macrocultures, and alterations in the ability of small numbers of cells to initiate microcultures. This *in vitro* assay is being used to determine the concentrations of psoralens and doses of UV-A which will inhibit DNA synthesis and cell proliferation. These studies suggest that the low doses of UV-A and 8MOP received by patients lymphocytes during therapy may be sufficient to explain the decreased DNA synthesis found in their circulating leucocytes.

Collaborative studies have begun using the alkaline elution assay to measure the contribution of DNA cross-linking to lymphoblastoid cell survival following UV-A plus psoralen.

Collaborative studies have begun to measure the induction of sister chromatid exchanges in human lymphoid cells following UV-A plus psoralen.

By using lymphoblastoid cells defective in DNA repair, e.g. from patients with xeroderma pigmentosum, the *in vitro* assay is being used to measure the contribution of DNA repair to the cellular and molecular effects of psoralen plus UV-A.

The observation was made that the ultraviolet light source used in the clinical "PUVA" therapy program in the absence of psoralens causes a significant decrease in DNA synthesis and inhibition of cell growth of human lymphoblastoid cells. Studies are being performed using optical filters to determine the portions of the spectrum causing this effect.

Publications

Kraemer, K.H., and Weinstein, G.W.: Decreased thymidine incorporation in circulating leucocytes after treatment of psoriasis with psoralen and longwave untraviolet light. J. of Invest. Dermatol. 69: 211-214, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-04517-02-CH

PERIOD COVERED
July 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

DNA Repair in Human Cancer-Prone Genetic Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: K.H. Kraemer   Research Scientist   CH NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Chemistry Branch, Carcinogenesis Program

SECTION
Cell Growth Regulation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.3	OTHER: 0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS   XX (b) HUMAN TISSUES   ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Radiation and carcinogen-induced DNA damage and repair in human cancer-prone genetic diseases is being studied. Attention is presently focused on xeroderma pigmentosum (XP), a disease with ultraviolet sensitivity, and ataxia telangiectasia (AT), a disease which recently has been found to exhibit X-ray sensitivity. A detailed comparison of the clinical features of these diseases was made and demonstrated remarkable similarities, particularly in the neurological abnormalities in both diseases. Cell fusion studies to demonstrate possible genetic heterogeneity of DNA repair defects among AT strains have begun. Cell fusion studies have also begun to attempt to detect persons heterozygous for XP or AT. Correlation of clinical features of XP and AT with in vitro DNA repair defects may lead to further understanding of the link between DNA repair and cancer.

PHS-6040
(Rev. 10-76)

Project Description

Xeroderma pigmentosum (XP) and ataxia telangiectasia (AT), two rare autosomal recessive diseases associated with high incidence of neoplasia, have been found to be very sensitive to radiation and to have defective DNA repair. Studies with XP fibroblast strains performed in the Dermatology Branch, NCI, using cell fusion techniques have demonstrated 5 different defects in repair of ultraviolet-induced DNA damage. The objective of this project is to verify that AT fibroblast strains are defective in DNA repair and to determine if genetic heterogeneity exists similar to that in XP fibroblasts. Autoradiographic and cell fusion studies have been initiated to measure DNA repair following exposure to AT cells to a potent carcinogen, nitrosoguanidine or to other DNA-damaging agents such as bleomycin.

A detailed comparison of the clinical features and cellular abnormalities in XP and AT was made. There were remarkable similarities, particularly in the neurological abnormalities in both diseases. Features common to both diseases may be the end result of extremely defective DNA repair in certain cell types or may indicate relatively greater local concentrations of DNA damaging factors. Correlation of clinical features of these diseases with in vitro DNA repair defects may lead to further understanding of the link between DNA repair and cancer.

Repair of DNA damage (strand breaks and release of bases) caused by the chemotherapeutic agent, bleomycin, is being studied in normal and repair defective human cells. These studies may lead to better understanding of the mechanism of cell recovery from defined DNA lesions.

Epidemiological studies of blood relatives of patients with AT and XP have suggested that asymptomatic carriers of these diseases have an increased incidence of neoplasia. Cell fusion studies have begun using haploid (sperm) cells to attempt to detect the (heterozygous) carrier state of XP or AT in vitro. Since heterozygotes for AT or XP probably occur in frequency greater than 1%, detection of this population may be an indicator of a large population with an increased risk of cancer.

Collaborative studies have begun of clinical features and DNA repair in patients with familial malignant melanoma.

Publications

Kraemer, K.H.: Progressive degenerative diseases associated with defective DNA repair: xeroderma pigmentosum and ataxia telangiectasia. In Nichols, W.W. and Murphy, D.G. (eds.) DNA Repair Processes. Miami Symposia Specialists, 1977, pp37-71.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04518-02-CH

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Anticodon: Codon Adaptation and Misreading of Amino Acyl-tRNAs in Protein Synthesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. Hatfield	Research Biologist	CH	NCI
	J.F. Mushinski	Medical Officer	CB	NCI
OTHER:	M. Rice	Chemist	CH	NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Chemistry Branch, Carcinogenesis Program

SECTION
Nucleic Acids Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
The purpose of this project is to determine if the concentrations of isoacceptor aa-tRNAs in rabbit reticulocytes are adapted to the usage of their corresponding codons in globin mRNA (i.e., anticodon:codon adaptation) and to determine if misreading of isoacceptor aa-tRNAs in protein synthesis may play a role in the development of cancer.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: To resolve isoacceptor aa-tRNAs from rabbit reticulocytes and determine the concentrations of each isoacceptor and to determine if isoacceptor aa-tRNAs which may be misread during protein synthesis may be involved in the expression of cancer.

Methods Employed: Transfer RNAs have been isolated from mammalian tissues, including rabbit reticulocytes, and isoacceptor aa-tRNAs have been resolved by multiple fractionations on reverse phase chromatographic columns to determine coding patterns and to determine concentrations. Human reticulocyte aa-tRNAs have been compared to those of rabbit reticulocytes by reverse phase chromatography. Transfer RNAs from wheat germ, yeast, E. coli, Hymenoptera, Xenopus liver, rabbit liver, and chicken liver are presently being aminoacylated under homologous and heterologous conditions to determine if mammalian synthetases may misrecognize heterologous tRNAs and to determine the extent of aminoacylation of each tRNA by aa-tRNA synthetases from heterologous organisms. If an isoacceptor is found which is efficiently misrecognized by mammalian aa-tRNA synthetases, this isoacceptor will be administered to mammalian cells in culture. Mammalian cells in culture are known to take-up tRNA from the media and efficient misrecognition of isoacceptor aa-tRNAs from different species has been demonstrated in other laboratories. Cross aminoacylation studies will provide an evolutionary record of the changes which have occurred in tRNA: synthetase interactions. Isoacceptor patterns of codon recognition of eleven aa-tRNAs from Hymenoptera and ten from wheat germ have been examined after fractionation of reverse phase chromatographic columns in a ribosomal binding assay.

Phe-tRNA which contains Wye base has been isolated from normal tissues and Phe-tRNA which lacks Wye base and is characteristic of malignant tissues, has been isolated from FrNTD and neuroblastoma cells by standard techniques and purified by successive chromatographic runs on reverse phase chromatographic columns. Codon recognition properties in a ribosome binding assay and the ability to transfer phenylalanine to protein in a cell free protein synthesis system of normal Phe-tRNA and Phe-tRNA minus Wye base have been carried out. The Phe-tRNA project is a collaboration with Dr. J.F. Mushinski.

Major Findings: Human reticulocyte and rabbit reticulocyte aa-tRNAs have similar chromatographic profiles for most aa-tRNAs. Pronounced differences in Asn-tRNA and His-tRNA profiles were observed between the two tissues. Whereas, most codons used in B-globin mRNA of both tissues are the same (Kafatos et al, Proc. Nat. Acad. Sci. 74: 5618, 1977), the frequency of codons used for these latter amino acids are significantly different. Rabbit liver Ala-$tRNA_{GCA}$ has been resolved from other Ala-tRNA isoacceptors and is more abundant in livers which have been treated with phenylhydrazine. Bovine liver Val-tRNA has

been resolved into an isoacceptor which recognizes GUA and isoacceptors which recognize GUU, GUC, GUA and GUG. The response to GUG relative to other codons varies considerably depending on the fraction used. Multiple fractionations of isoacceptor aa-tRNAs on reverse phase columns have proven not to be a good technique for determining concentrations of specific isoacceptors. Alternative methods which will separate isoacceptors in a single step must be obtained in order to determine accurately concentrations of specific isoacceptor aa-tRNAs in reticulocytes.

Patterns of codon recognition of Hymenoptera isoacceptor aa-tRNAs were similar to those previously observed from mammalian tissues. The relative abundances of several isoacceptor aa-tRNAs, however, are significantly different between the two organisms. Differences in the patterns of codon recognition of isoacceptor aa-tRNAs from Hymenoptera and wheat germ were observed and the relative abundances of several isoacceptors recognizing the same codewords were different between the latter two organisms.

Codon recognition properties of normal Phe-tRNA and Phe-tRNA from neoplastic tissues were indistinguishable with regard to wobble in the 5', 3' or middle positions. Neuroblastoma Phe-tRNA is deacylated more slowly than normal Phe-tRNA in a protein synthesis system. The possibility that Phe-tRNA from neoplastic tissues may be misread in protein synthesis is presently being carried out. (This project is a collaboration with Dr. J.F. Mushinski.)

Significance to Biomedical Research and the Program of the Institute: A major unresolved question in biology is whether tRNA may play a role in cellular regulation and carcinogenesis. Approaches to elucidating these problems are to determine if the levels of isoacceptor aa-tRNAs are adapted to the requirements of protein synthesis; and if misreading of isoacceptor aa-tRNAs in protein synthesis can induce cell transformation.

Proposed Course of Project: To complete the cross aminoacylation studies, to isolate isoacceptor aa-tRNAs from heterologous sources which may be misrecognized by mammalian synthetases for administering to cells in culture and to determine if Phe-tRNA minus Wye base may transfer its amino acid into protein sites not coded by phenylalanine.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CP 04525-06 CH

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Studies on Electrophoretic Techniques for Protein, RNA, and DNA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: A. C. Peacock Head, Protein Section CH NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Chemistry Branch, Carcinogenesis Research Program

SECTION

Protein Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	0.9	1.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A new method for estimation of chain lengths of ribonucleic acids is under development. The method involves partial heat degradation of the RNA under study and measurements of the remaining and degraded molecules. From similar measurements on reference RNAs, the chain lengths may be deduced. In addition, computer simulation permits computation of the relation between chain length and electrophoretic mobility for use in electrophoretic analysis of polydisperse samples. Heterogeneous nuclear RNA from HeLa cells has been studied by these techniques.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04525-06-CH

Project Description

Objectives: To investigate the influence of primary structure and conformation on the electrophoretic and biological properties of proteins and nucleic acids and to use this information in devising improved methodology for analysis and purification. To study the role of hnRNA in cellular control in normal and malignant cells by these techniques.

Methods Employed: Cultures of HeLa cells, assay of radioisotopes, isolation and electrophoresis of RNA and DNA, optical scanning of stained and unstained gels, preparative electrophoresis, optical measurement of melting of nucleic acids, ultracentrifugal analysis of RNA and DNA.

Major Findings: The validity of the thermal degradation method for size estimation has been established for mammalian ribosomal RNAs and RNA from the virus MS2. In addition, the (known) relationship between length and electrophoretic mobility was the same as that found by computer analysis of the thermal degradation data.

Heterogeneous nuclear RNA (hnRNA) prepared using DMSO as described earlier was studied by these methods. The most convenient material was derived from HeLa cells which had been treated with actinomycin D to suppress synthesis of ribosomal RNAs. The hnRNA was found to be smaller and more homogeneous than had been indicated earlier. The modal length was approximately 15,000 nucleotides (Mwt: 5.1×10^6); the weight average length was 1×10^4 nucleotides; the number average length was 0.6×10^4 nucleotides.

The electrophoretic analysis could be made without the necessity of repeating the thermal degradation by means of a calibration table permitting the use of the ribosomal RNAs as markers.

Significance to Biomedical Research and the Program of the Institute: The ability to estimate chain lengths of polydisperse ribonucleic acids by methods dependent on molecular properties very different from those previously available (centrifuge, electron microscope, etc.) provides a check on these methods. It also permits modifications in sample preparation, a step which may introduce ambiguities.

The heterogeneous RNA is the primary transcription of the gene and, in addition to specific messengers for specific proteins, presumably contains all RNA that might be involved in the control of genetic expression. Analytical methods for studying this RNA and assessing its role may be fundamental to understanding the alterations which occur in malignancy.

Proposed Course of Project: It is hoped to complete the analysis of the major factors that determine electrophoretic mobility of hnRNA and subsequently to examine this material in a variety of cell types and states.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04554-04-CH

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Development of the System for Assay of In Vitro Chemical Carcinogenesis Using Human and Mouse Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Kakunaga	Head, Cell Growth Regulation Section	CH	NCI
OTHER:	J. Crow	Biologist	CH	NCI

COOPERATING UNITS (if any)

NONE

LAB/BRANCH
Chemistry Branch, Carcinogenesis Program

SECTION
Cell Growth Regulation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.6	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to establish the system of assay of in vitro malignant transformation of human and mouse cells by chemical carcinogens. As a continuation of our previous success in the transformation of human cells, quantitation and earlier detection of the transformation of human cells after chemical treatment have been attempted. Abortive or transient transformation was observed for one to three months after treatment with 4-nitroquinoline-1-oxide. Treatment with a promotor during one month after 4NQO-treatment enhanced the frequency and shortened the time of the development of abortive transformation. However, treatment with the promotor did not result in the enhancement of stable and malignant transformation. A quantitative system for assay of malignant transformation using a subclone of BALB/3T3-A31 clone was improved and was given to many outside laboratories.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: To establish a quantitative system for assay of *in vitro* chemical transformation of human cells.

Methods Employed: Diploid human fibroblast cells are obtained from biopsies taken from skin of a normal adult female. The cells are grown as a mass culture under a culture condition using a selected lot of serum which gave regularly a cloning efficiency as high as 40-80%. The cells are exposed to chemical carcinogens and some groups of them are then repeatedly treated with a promotor, 12-O-tetradecanoyl phorbor-13-acetate (TPA).

The cells are examined for the morphology and the ability to grow in soft agar. Morphology of the cells is examined by microscopy before and after fixation and staining. Cells which show altered morphology or growth patterns are picked up and tested for their ability to grow in soft agar. The colonies formed in soft agar are picked up and then retested for their growth in soft agar and are also examined for their morphology when cultured in liquid medium.

A mouse cell line, A31-I variant previously isolated in this section, is used as a target cell for a quantitative system for assay of neoplastic transformation by chemical carcinogens. Assay is processed according to the procedure previously reported with A31-714 cell (Kakunaga, *Int. J. Cancer*, *12*: 463-473, 1973).

Major Findings: In order to quantitate and enhance the frequency of the transformation of normal human cells after treatment with chemical carcinogens, soft agar method and TPA were used. The cell which can grow in soft agar ("agar$^+$ " cells) were first detected about one month after chemical treatment and then increased in number with continuous subcultivation. However, the number of agar$^+$ cells reached its maximum at two months after chemical treatment, and then gradually decreased. When the colonies in soft agar were picked up from these early cultures and were cultured in liquid medium, morphology of agar$^+$ cells were not markedly altered except for a little higher cell density compared to normal or agar- cells. In total, more than 300 colonies were picked up from soft agar culture into which the cells were plated within 3 months after chemical treatment, and they were repeatedly tested for the ability to grow in soft agar. Only two of them proved to be stable in their ability to grow in soft agar. Thus, most of agar$^+$ cells were considered to be the result of abortive or transient transformation. Treatment with a promotor during one month after 4NQO-treatment enhanced the frequency and shortened the time of the abortive transformation. However, the treatment with the promotor did not enhance stable transformation.

A quantitative system for assay of malignant transformation using a subclone of BALB/3T3-A31-I clone was improved mainly by selecting a new subclone which does not require strictly for quality of serum to develop the transformation.

This subclone has been distributed to many outside laboratories with detailed information and instructions on the assay procedure, culture condition, and properties of the cells.

Significance to Biomedical Research and the Program of the Institute: To date there has been no reproducible system established in which normal diploid human cells are transformed *in vitro* by chemicals. Rationale for the establishment of such a system is as sound as any at this time. This project will provide a system in which to assay potential carcinogens directly on human cells, thus removing the necessity of extrapolating from data obtained from animal cells. It would also provide important information on the relationship of the response to the carcinogenic effect of chemical compounds between the cells from different species and between *in vivo* and *in vitro* results. The information thus obtained would be very helpful for extrapolating the result in animal experiments or in *in vitro* system into assessment of human risk to environmental carcinogens.

Proposed Course of Project: To pursue the goals as outlined above in Objectives and to publish any results so obtained.

Publications

Kakunaga, T.: The Cell Transformation of Human Diploid Cells by Chemical Carcinogens. In Hiatt, H.H., Watson, J.D. and Winsten, J.A. (ed.): *Origins of Human Cancer*. New York, Cold Spring Harbor Laboratory, 1977, pp 1537-1548.

Kakunaga, T.: Chemical Transformation of Human and Roden Cells. In Soffiotti, U. and Autrup, H. (ed.): *In Vitro Carcinogenesis*. NCI Technical Report Series No. 44, HEW, 1978, pp 84-99.

Kakunaga, T.: Neoplastic Transformation of Human Diploid Fibroblast Cells by Chemical Carcinogens. *Proc. Natl. Acad. Sci. USA*. In press, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-CP-04555-04-CH

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Factors Affecting Malignant Transformation by Chemical Carcinogens in Human and Mouse Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Kakunaga	Head, Cell Growth Regulation Section	CH	NCI
OTHER:	J. Crow	Biologist	CH	NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Chemistry Branch, Carcinogenesis Program

SECTION
Cell Growth Regulation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.3	0.6

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range purpose of this work to clarify the mechanism of cell transformation by studying the genetic and biological factors affecting carcinogenesis by chemicals. This work also aims to clarify the role of these factors in the etiology of human cancer incidence. The topics of present interest are: 1) the frequency of chemical transformation of the cells derived from xeroderma pigmentosum patients who are predisposed to a higher incidence of cancer; and 2) the characterization of cell variants which shows the different sensitivity to chemical transformation.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: To learn more about the mechanisms involved in the malignant transformation of cells in vitro and to determine more appropriate systems for the in vitro assay of chemical carcinogens.

Methods Employed: Two systems of cell transformation by chemical carcinogens were used: 1) A system of neoplastic transformation of human diploid fibroblast derived from skin biopsy which was developed in this laboratory (see Project No. Z01-CP-04554-03 & 04-CH). 2) A quantitative system for the assay of malignant transformation of subclones derived from BALB/3T3-A31 clone which was previously developed (see Project No. Z01-CP-04555 series).

Major Findings: 1) It was confirmed that the fibroblast cells derived from a xeroderma pigmentosum (XP) patient were extremely (classical XP cells) or moderately (variant XP cells) sensitive to the cytotoxic effect of carcinogens. Attempts to transform XP cells by treatment with 4-nitroquinoline-1-oxide have resulted in observation of only one morphologically altered colony. It is suggested that the transformation frequency of XP cells may not be higher than that of normal cells when the frequency is calculated on the basis of the number of cells treated. The comparative study of the transformation frequency per survived cells after 4NQO-treatment between normal and XP cells is being continued.

2) Cell variants previously isolated from a BALB/3T3-A31 clone were classified into four groups according to their different sensitivities to the chemical carcinogens. There was no significant difference in the doubling time and saturation density among these variants.

Significance to Biomedical Research and the Program of the Institute: The results from this project will provide: 1) A useful system for the analysis of the mechanism of chemical carcinogens. 2) Information about the mechanism of cell transformation by chemical carcinogens. 3) Optimum conditions needed for the assay of potential chemical carcinogens using human and mouse cells. 4) Basic information about the etiological factors in the incidence of human cancer. Thus, this project is directly aimed at the aspect of the Program of the Institute which seeks to find rapid meaningful assays for potential chemical carcinogens.

Proposed Course of Project: To pursue the goals as outlined in Objectives and to publish any results so obtained.

Publications

Kakunaga, T.: Factors affecting the Polycyclic Hydrocarbon-Induced Cell Transformation. In Ts'o, P.O.P. and Gelboin, H.V. (ed.) Polycyclic Hydrocarbons and Cancer. New York, Academic Press, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04563-05 CH

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Studies with Cationic Carbocyanine Dyes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: M. R. Green Research Chemist CH NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Chemistry Branch, Carcinogenesis Research Program

SECTION
Protein Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The dye 1-ethyl-2-[3-(1-ethylnaphtho[1,2d]thiazolin-2-ylidene)-2-ethylpropenyl]naphtho[1,2d]thiazolium bromide is a metachromatic dye of unusual sensitivity for differentiating various classes of macromolecules by color. Methods for staining proteins, phosphoproteins, sialoglycoproteins, glycosaminoglycans and nucleic acids differentially in tissue sections and on polyacrylamide gels following electrophoresis were developed. These methods were applied to the study of milk and breast tissue proteins. A histochemical study of normal and neoplastic human breast tissue was done and the macromolecules listed above were identified.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04563-05-CH

Project Description

Objectives: To obtain more information about the characteristics of the dye 4,5,4',5'-dibenzo-3,3',9 triethyl thiacarbocyanine bromide also known as 1-ethyl-2-[3-(1-ethylnaphtho[1,2d]thiazolin-2-ylidene)-2-ethylpropenyl]naphtho-[1,2d]thiazolium bromide and its interaction with macromolecules. Identification of acidic macromolecules on gels and tissue sections by metachromatic reactions and histochemical procedures.

Methods Employed: Polyacrylamide gel electrophoresis, staining and scanning of gels by spectrophotometry, absorption measurements of dye solutions by recording spectrophotometry, observation of color reactions of pure substances in films and solutions, enzymatic digestions and solvent extractions of tissue sections, microscopic observation of stained sections and cells in tissue culture.

Major Findings: The cationic carbocyanine dye, Catalog #2718, labeled 1-ethyl-2-[3-(1-ethylnaphtho[1,2d]thiazolin-2-ylidene)-2-methylpropenyl]naphtho[1,2d]-thiazolium bromide, Lot 711-1, (Stains-all) was obtained from Eastman Kodak Co., Rochester, N. Y., beginning in 1971. This lot was used throughout this study and in papers published from this laboratory from 1973 to the present time. The published studies were concerned with the histochemical identification of macromolecules on gels and in tissue sections and the dye was referred to as Stains-all.

Shortly before publication of this report the supply of Lot 711-1 was depleted. Other lots of Stains-all were used; these gave results which differed substantially from those described earlier. The company stated that the compound in bottles labeled Lot 711-1 was not 1-ethyl-2-[3-(1-ethylnaphtho[1,2d]thiazolin-2-ylidene-2-methylpropenyl]naphtho[1,2d]thiazolium bromide (Stains-all) or by another nomenclature 4,5,4',5'-dibenzo-3,3' diethyl 9 methyl thiacarbocyanine bromide (DBTC), but was 1-ethyl-2-[3-(1-ethylnaphtho[1,2d]thiazolin-2-ylidene-2-ethylpropenyl]naphtho[1,2d]thiazolium bromide (Ethyl-Stains-all) or 4,5,4',5'-dibenzo-3,3' 9 triethyl thiacarbocyanine bromide (DBTC-3,3' 9 triethyl). NMR analysis confirmed that Lot 711-1 was the triethyl compound and that staining and solubility properties of DBTC-3,3' 9 triethyl matched Lot 711-1, while DBTC matched other lots of Stains-all.

The simultaneous differential staining of phosphoproteins, sulfated glycosaminoglycans, hyaluronic acid, sialoglycoproteins, and nucleic acids was demonstrated in surgical specimens of normal and malignant human breast tissue fixed in formalin and embedded in paraffin using the dye 1-ethyl-2-[3-(1-ethylnaphtho-[1,2d]thiazolin-2-ylidene)-2-ethylpropenyl]naphtho[1,2d]thiazolium bromide (Ethyl-Stains-all), a metachromatic cationic carbocyanine dye. This simple staining method gives information concerning the types of macromolecules present in connective tissue and in secretions within cells, alveolar lumina and ducts. Nuclear counterstaining is not required. Enzyme digestions confirmed the presence of the various macromolecules listed. Comparison with Alcian Blue, Periodic Acid Schiff or Toluidine Blue staining indicates that Ethyl-Stains-all has advantages in terms of simplicity, specificity and sensitivity for the detection of these macromolecules in pathologic material.

Studies of milk and breast tissue proteins included analyses of colostral milk from mice and humans and membrane proteins of mice.

Histochemical studies of various types of mouse mammary tumors, types A, B, C (Dunn classification) are in progress.

Significance to Biomedical Research and the Program of the Institute: This stain is particularly useful for detection of sialoglycoprotein production by normal and malignant tissue and for showing the presence of hyaluronic acid and sulfated glycosaminoglycans in stromal tissue. It would be of interest to correlate the presence of sialoglycoprotein in the primary tumor with the presence in serum of sialoglycoproteins (CEA, κ casein, β subunit of HCG, α fetoprotein and other breast-specific sialoglycoproteins) in patients with recurrent metastatic disease. If such a correlation could be made it might help in selecting patients for radioimmunoassay testing. The methods developed are useful for identification of different classes of macromolecules in small samples, on gels following electrophoresis, or in tissue sections.

Proposed Course of Project: The histochemical methods will be applied to cytologic and biopsy specimens to determine whether they have special utility for the detection of early neoplastic lesions. Normal and neoplastic cells in culture will be studied to determine whether the production of certain macromolecules can be correlated with neoplastic transformation.

Publications

Green, M. R.: Simultaneous differential staining of phosphoproteins, sialoglycoproteins, hyaluronic acid, sulfated glycosaminoglycans, proteins and nucleic acids in human breast tissue. J. Nat. Cancer Inst. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04578-02-CH

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Genes Responsible for the Transformed Phenotype in Chemically Transformed Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. Ohki	Visiting Fellow	CH	NCI
OTHER:	T. Kakunaga	Head, Cell Growth Regulation Section	CH	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Chemistry Branch, Carcinogenesis Program

SECTION
Cell Growth Regulation Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 1.2	PROFESSIONAL: 1.1	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the purpose of this work to clarify the nature of molecules responsible for converting a normal cell to its malignant form by studying the nature of heat-sensitive (hs) or cold-sensitive (cs) mutants of Chinese hamster lung cells affected in the expression of the transformed state. As one of the approaches to the above purpose, it was examined whether there is complementation between hs-mutants or cs-mutants, which have previously been isolated by us. The resistance to ouabain or 6-thioguanine was introduced into these mutants as a selective marker and many cell hybrids were obtained from two different mutants using techniques of cell fusion and selective growth. Examination of temperature-dependency to express the transformed phenotype and karyotype of the cell hybrids suggest that there is a complementation in the expression of the transformed state at non-permissive temperature between hs-mutants.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: To learn about genetic information on the expression of transformed phenotype, and to obtain the clues for biochemical approach to the molecules controlling the transformed phenotype.

Methods Employed: In ts-mutants, permissive temperature of the incubation is 34.5°C and non-permissive temperature is 39°C for the incubation. Permissive temperature is 30°C and non-permissive temperature is 34.5°C in cs-mutants. Ability of the cells to grow in soft agar (*Int. J. Cancer* 12: 463-473) is used as a transformed phenotype because of its close correlation to the *in vivo* malignancy of the cells, and because of its more objective process to diagnose this criteria compared to other criteria of the transformed phenotype.

A cell hybridization technique is used for examining the complementation group of mutants. In order to select out the hybrid cells from nonhybrid cell population in this experiment, markers like a resistance to 6-thioguanine and/or ouabain is introduced into all mutants. Polyethylene glycol is used to induce the cell fusion. Optimum experimental conditions are determined to obtain 1:1 hybrid cells at high frequency.

Major Findings: More than one hundred colonies which were formed in the presence of ouabain and 6-thioguanine and were supposed to be composed of hybrid cells were isolated after fusing two different cell mutants. Examination of modal chromosome numbers showed that most of the cell lines thus obtained were hybrid cells. Several lines had modal chromosome numbers similar to parental mutant cells and were considered that they might be spontaneously mutated cells among cell population of parental lines.

The hybrid cells showed considerable variations in the sensitivity to the temperature of incubation to grow in soft agar gel. About half of the hybrids obtained from two different hs-mutants lost their sensitivity to the temperature, whereas the rest retained their parent s trait, heat sensitivity. Preliminary examination of chromosomal numbers showed that their karyotype is very unstable and that some parts of chromosomes are easily lost during cultivation after cell fusion. It is likely that the hybrid which retains a complete set of both parent chromosomes gained their ability to express the transformed phenotype at the temperature which is non-permissive for parent mutants. Segregation of some chromosomes from hybrid cells may result in the loss of their ability to express the transformed phenotype at the higher temperature.

Significance to Biomedical Research and the Program of the Institute: The results from this project will provide: 1) A useful system for the anaysis of the transformed phenotype; 2) The information about genes controlling the expression of the transformed state; and, 3) Information on the nature of the molecules responsible for converting a normal cell to a malignant cell. Thus, this project is directly aimed at that aspect of the Program of the

Institute which seeks to find an essential difference in the biochemical nature between normal and malignant cells.

Proposed Course of Project: To pursue the goals as outlined in Objectives and to publish any results so obtained.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04579-02 CH

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Benzo(a)pyrene and (-)trans-7,8-diol Metabolism by Highly Purified Forms of Cytochrome P-450.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Deutsch	Visiting Scientist	CH NCI
OTHER:	H. V. Gelboin	Chief, Chemistry Branch	CH NCI
	J. C. Leutz	Chemist	CH NCI
	M. J. Coon	Professor	U. of Michigan School of Medicine Ann Arbor, Michigan

COOPERATING UNITS (if any)
Department of Biological Chemistry, University of Michigan School of Medicine, Ann Arbor, Michigan

LAB/BRANCH
Chemistry Branch, Carcinogenesis Research Program

SECTION
Molecular Carcinogenesis

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.2	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long range purpose of this project is to study the mechanism of the activation and detoxification of benzo(a)pyrene by mixed function oxidase. For this purpose highly purified cytochrome P-450's from rabbit liver in a reconstituted system were used for the metabolism and DNA and protein binding of benzo(a)pyrene and (-)trans-7,8-diol.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: To determine the metabolism of carcinogenic polycyclic hydrocarbons by purified forms of cytochrome P-450, the principal enzymes involved in carcinogen activation and detoxification.

Methods Employed: Fluorospectrophotometry, high pressure liquid chromatography, mass spectrometry, slab gel electrophoresis and radiography.

Major Findings: Highly purified cytochrome P-450's from rabbit liver in a reconstituted system exhibit different catalytic activities in the metabolism of benzo(a)pyrene and BP(-)-trans-7,8-diol. The different cytochrome P-450's also exhibit differences in the activation of BP and (-)trans-7,8-diol to highly mutagenic and carcinogenic diol epoxide I and II.

Significance to Biomedical Research and the Program of the Institute: The analysis of the selectivity of the multiple cytochrome P-450 forms is an essential step toward the understanding of chemical carcinogen action. The unique catalytic activity of different cytochrome P-450's may be the basis between activation and detoxification pathways of benzo(a)pyrene. This finding is an essential step toward the understanding of chemical carcinogen action, and may help in the modification of the body's defense against chemical carcinogens.

Proposed Course of Project: To identify and quantitate the benzo(a)pyrene metabolites formed by the multiple forms of cytochrome P-450. To determine the role of the enzyme forms in the biological effects of benzo(a)pyrene in toxicity, mutagenicity and carcinogenicity.

Publications

Deutsch, J., Leutz, J. C., Yang, S. K., Gelboin, H. V., Chiang, Y. L., Vatsis, K. P. and Coon, M. J.: Regio- and stereoselectivity of various forms of purified cytochrome P-450 in the metabolism of Benzo(a)pyrene and (-)trans-7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene.as shown by product formation and binding to DNA (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04782-08 CH

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Hormones and Breast Tissue Interactions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. R. Green	Research Chemist	CH NCI
OTHER:	A. C. Peacock	Head, Protein Section	CH NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Chemistry Branch, Carcinogenesis Research Program

SECTION
Protein Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	0.7	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to contribute to the understanding of hormone action on metabolic processes in normal breast tissue under various conditions and to understand the failure of or aberrant response to hormones by hyperplastic or neoplastic breast tissue under the same conditions. Among the topics studied are the response of mammary tissue in culture to hormones in terms of nucleic acid, protein, glycoprotein and glycosaminoglycan synthesis. The kinds of proteins made by mammary tissue from normal non-pregnant, mid-pregnant, lactating animals and by neoplastic mammary tissue are being analyzed. Response to prolactin and steroid hormones is assessed by chemical and histochemical methods. Histidine requiring mutant strains of Salmonella typhimurium are used for detecting mutagenic metabolites.

PHS-6040

Project No. Z01-CP-04782-08-CH

Project Description

Objectives: To gain a clearer understanding of the interaction of hormones with breast tissue. The topics of interest are interaction of hormones such as insulin, hydrocortisone and prolactin with breast tissue components and metabolism of steroids by breast tissue. Responses by breast tissue to prolactin and steroids in terms of nucleic acid synthesis and synthesis of macromolecules such as phosphoproteins, sialoglycoproteins, glycosaminoglycans and proteins are under investigation as are modulation of these responses in mouse mammary tumor virus containing tissues and in neoplastic tissue. The hypothesis that estrogen metabolites may act as mutagens is being tested using Salmonella tester strains developed by B. Ames.

Methods Employed: Culture of mammary tissue explants *in vitro* using defined medium, isolation and characterization of nucleic acids, separation on agarose-acrylamide gels and on gradients, radioactive tracer techniques, isolation and purification of proteins from milk and explants, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, histochemical analysis of tissue sections, autoradiography, binding of hormones to macromolecules, mutagenesis assay using Salmonella tester strains.

Major Findings: A bioassay for prolactin was reported based on the finding that the amount of caseins present in proteins extracted from mouse mammary explants after five days of incubation on defined medium containing insulin, hydrocortisone and 0 to 100 ng/ml of prolactin was a measure of the prolactin concentration in the medium. Densitometric analysis of explant proteins stained following electrophoresis on SDS-polyacrylamide gels indicated that most proteins were present in relatively constant proportion as prolactin concentration in the medium increased; exceptions were the caseins and a protein near the lactoperoxidase marker, 93,000 M.W. It seemed that this protein could also be one of the milk-specific proteins. This protein has now been identified. It is a glycoprotein that binds iron in a system that lacks the serum iron-binding glycoprotein, transferrin. Its migration in several electrophoretic systems is the same as lactoferrin. On SDS gels it migrates with the same mobility as mouse, bovine and human lactoferrin. Finally, a semi-purified fraction containing the responsive protein gives a precipitin reaction with rabbit antiserum to purified mouse lactoferrin.

A series of investigations designed to test the hypothesis that certain metabolites of estrogens may cause mutations and bind to DNA was continued. Estrogens and 7,12-dimethylbenzanthracene were tested with and without S-9 supernatants from Sprague-Dawley rats using the Ames test. The results indicated that estrogens may produce about twice the number of revertants than control plates with S-9 alone. Statistical evaluation of the results obtained to date showed a highly significant difference between controls with S-9 and no compound and S-9 plus compound. These results need confirmation by further experimentation.

Significance to Biomedical Research and the Program of the Institute: The bioassay that was developed will be a useful adjunct in the evaluation of

biological activity of prolactins which are positive by radioimmunoassay but differ in size and charge and may lack biological activity. It may also provide information as to which portion of the prolactin molecule is required for biological activity and/or receptor binding.

The identification of lactoferrin as a prolactin-responsive protein adds yet another marker for the identification of cells in culture as well as for testing biopsies and cells in culture for prolactin response.

In the past few years several laboratories have identified proteins of the mouse mammary tumor virus. This laboratory has been engaged in the characterization and identification of the major mouse milk proteins, α-lactalbumin, κ casein and three other caseins, albumin and lactoferrin. This has been done so that the interplay of hormones on protein synthesis of normal and virally infected mammary tissue may be studied in greater detail. The hope is that modifications in breast tissue constituents following viral infection that lead to tumor formation can eventually be dissected out and understood.

The influence of hormones on human breast cancer has been the subject of intensive investigation. Epidemiologic investigations of recent years have tended to reinforce the idea that genetic and environmental factors enter heavily into this disease process. An increased association has been shown among individuals migrating from the Orient to Hawaii and then to the United States. Dietary factors and obesity as well as age at first pregnancy play an important role. Since metabolic processes seem implicated, one wonders whether under unfavorable circumstances hormones of a certain class may become carcinogens. Estrogens given over long periods of time in large quantities have proved carcinogenic in animals. The association in humans is less clear, however, estrogens are thought to act primarily by influencing proliferation of cells damaged by other means, *i.e.*, radiation, viruses or chemicals. The Sprague-Dawley rat has been the animal of choice for the study of DMBA mammary tumorigenesis. Prior to this time it was known that this strain was subject to spontaneous mammary cancer. The understanding of the molecular basis of binding of carcinogens like BP and DMBA to DNA is increasing at a very rapid pace. Since there are similarities in molecular structure to steroids, it becomes of great interest to reinvestigate whether estrogen metabolites can act as mutagen using newer, more sensitive techniques as exemplified by the Ames assay.

Proposed Course of Project: Studies of synthetic processes of normal, MMTV-infected and neoplastic mammary explants as influenced by hormones *in vitro* will continue.

The interaction of steroids, such as estrogens, and possible estrogen precursors and metabolites following metabolic activation by liver and breast fractions with DNA will be assessed by using the *Salmonella* revertant strains. Enzymatic methods other than those present in microsomes will be used to produce the suspected metabolites. Radioactively labeled steroid binding to DNA *in vitro* will be studied.

Publications

Green, M. R., Pastewka, J. V., and Peacock, A. C.: Bioassay for prolactin: Densitometric analysis on polyacrylamide gels of milk protein production by mammary explants in vitro. Endocrinology 101: 1784-1791, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04785-08-CH

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

The Role of DNA Repair Mechanisms in the Etiology of Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.S. Day, III	Research Physical Scientist	CH	NCI
OTHER:	C. Ziolkowski	Chemist	CH	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Chemistry Branch, Carcinogenesis Program

SECTION
Nucleic Acids Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The previously developed adenovirus host-cell plaque assay (in which adenovirus 2 or 5 is treated with physical or chemical agents, and is then assayed for biological activity on monolayers of human cells) was used in three studies: 1) Fibroblasts from persons afflicted with Cockayne's syndrome were found less able than fibroblasts from apparently normal persons to repair DNA damage due to ultraviolet light; 2) ultraviolet light was found to cause mutations (reversions) in an adenovirus 5 temperature sensitive mutant; and 3) N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) treatment of human kidney carcinoma cells was found to decrease their capacity to repair MNNG treated adenovirus 5.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: To learn more about DNA repair mechanisms in human cells and about their role in carcinogenesis. In particular, to determine the nature of the defects in cells from persons who are genetically predisposed to cancer. In addition, to use human cell strains with characterized defects to study the mechanisms of action of carcinogens or suspect carcinogens in altering DNA.

Methods Employed: Using an adenovirus-host cell reactivation assay developed previously in this project, it has been possible to quantitate the deleterious effects of various chemical and physical treatments on the ability of the virus to initiate and sustain infection. The method involves establishing monolayer fibroblast cultures which are then infected with treated adenovirus. The infected cells are then incubated 12-20 days with feeding by means of periodic overlaying with a nutrient agar. Non-treated virions or those treated ones which have been "reactivated" by cellular repair mechanisms form countable plaques of dead, lysed cells. The plaquing efficiency of unirradiated adenovirus is approximately the same on all of the non-transformed human cell strains so far tested. It is 5 to 100-fold greater when human tumor lines of various types are used. Neutral and alkaline sucrose gradients are used to analyze the size of DNA extracted from treated adenoviruses, and agarose-polyacrylamide composite gels are used to analyze DNA for breaks and crosslinks.

Major Findings: 1) Studies concerning a DNA repair defect in fibroblasts from two persons having Cockayne's syndrome showed that about 80% of the cells from either patient were deficient in being able to repair ultraviolet light damaged adenovirus 5, whereas about 20% showed normal repair. This difference does not seem to be due to a patient's having a mixture of repair deficient and proficient cells since two clones grown up from these fibroblasts show similar behavior.

2) The effect of ultraviolet light on the reversion rate of adenovirus 5ts2 (a temperature-sensitive mutant of adenovirus 5, which grows at 32°, but not at 39°C) to temperature independent growth has been studied. A human kidney carcinoma cell line, A498, was selected from among 25 strains for this purpose. This line gives a 20-fold higher estimate of plaque forming units with a given adenovirus preparation than do normal human fibroblast strains, resulting in increased sensitivity of the assay. A498 cells grow well at 39°C, whereas no normal human fibroblast strain tested has done so consistently. A498 shows a marked decline in growth rate upon cell-cell contact, so that, unlike many other tumor lines tested, it does not deplete its nutrient supply to a lethal extent during the plaque assay. For the reversion studies, a preparation of adenovirus 5ts2 is irradiated and passaged once (in parallel with the non-irradiated control) through human fibroblasts at 32°C. The viral yields from this passaging are then analyzed for plaque

forming ability using A498 cells (to measure total plaque forming units) and 39°C (to measure revertant, temperature independent plaque forming units). In the study, the ratio of plaques at 39° to those at 32°C increased from 10^{-6} at zero ultraviolet dose to the virus to 10^{-4} when the viruses were irradiated with $800 J/m^2$ of germicidal ultraviolet before being passaged through normal human fibroblasts. In practice, this mutation assay should prove adaptable both to the study of different viral inactivating agents, and to the study of cells from humans genetically predisposed to cancer.

3) Treatment of A498 cells with the carcinogen MNNG (N-methyl-N - nitro-N-nitrosuguanidine) results in a decrease in their ability to support the growth of MNNG treated (but not of untreated) adenovirus 5. The MNNG treatment of the cells appears to decrease their ability to repair the MNNG treated viruses by 50%. The effect is very rapid: The depression of repair is complete even if cells are MNNG treated up to 3 hours after the start of infection by the MNNG treated adenoviruses. The MNNG doses required are quite toxic to the cells in terms of their ability to divide after the treatment, consistent with the idea that the intra-cellular depression of repair detected with the aid of the adenoviruses does have biological consequences to the cells themselves. This is the first evidence that a carcinogen may enhance the effects of its own damage by altering DNA repair mechanisms.

Significance to Biomedical Research and the Program of the Institute: Physical, chemical, and viral carcinogens are all known to alter the structural integrity of the cellular genetic apparatus. An evaluation of the role of DNA repair and/or related mechanisms in conferring resistence or susceptibility to mutagenesis and carcinogenesis is an important facet in any overall program having as its goal the understanding of the molecular pathways which, when perturbed, give rise to carcinogenesis in humans. It is the long range goal of this project to determine whether or not the elucidation of genetic repair mechanisms is important to the understanding of carcinogenesis. It is expected that an understanding of genetic repair mechanisms in general will benefit many areas of biomedical research.

Proposed Course of Project: To pursue the goals outlined above in Objectives and to publish these results.

Publications

Day, Rufus S., and DiMattina, M.: Photodynamic action of chlorpromazine on adenovirus 5: Repairable damage and single-strand breaks. Chemico-Biological Interactions 17: 89-97, 1977.

Day, Rufus S., III, UV-induced alleviation of K-specific restriction of phage lambda. Journal of Virology 21: 1249-1251, 1977.

Day, Rufus S., III, Scudiero, D. and DiMattina, M.: Excision repair by human fibroblasts of DNA damaged by 4-7, t-8-dihydroxy-t-9,10,-oxy-7,8,9,10 tetrahydrobenzo(a)pyrene. (Mutation Res. in the press.)

Day, Rufus S., III,: Human adenoviruses as DNA repair probes. in DNA Repair Processes (Cellular Senescence and Somatic Cell Genetics Series,) W.W. Nichols and D.G. Murphy, eds. Symposia Specialists, Miami. 1977 pp 119-145.

Day, Rufus S., III: Inducible error-prone repair and cellular senescence. in DNA Repair Processes (Cellular Senescence and Somatic Cell Genetics Series, W.W. Nichols and D.G. Murphy, eds. Symposia Specialists, Miami. 1977 pp217-223.

Day, Rufus S., III, Scudiero, D., and DiMattina, M.: Repair of DNA damaged by benzo(a)pyrne diol-epoxide I. in Polycyclic Hydrocarbons and Cancer: Environment, Chemistry, Molecular and Cell Biology, H.V. Gelboin and P.O.P. Ts o, eds., Academic Press (in press).

Day, Rufus S., III, and Ziolkowski, C.: Studies on UV-induced viral reversion, Cockayne's syndrome, and MNNG damage using adenovirus 5. in DNA Repair Processes. P.C. Hanawalt and E.C. Freidberg eds. (in the press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-5000-01-CH

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
DNA Repair Deficiency and Increased Probability of Cancer in Certain Human Genetic Diseases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: D.A. Scudiero Senior Staff Fellow CH NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Chemistry Branch, Carcinogenesis Program

SECTION
Nucleic Acids Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.4	2.4	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study chemical carcinogen-induced DNA repair in cell strains derived from patients with various genetic diseases showing an enhanced frequency of malignancy. Such investigations can determine if these cells have deficiencies in their ability to repair DNA damage and if such deficiencies can be correlated with the increased probability of cancer in these patients. The role played by DNA repair mechanisms in an organism's ability to tolerate certain levels of DNA damage can be important in assessing the relative carcinogenicity of particular compounds. Also, these studies can yield information about different classes of DNA-lesions induced by chemical carcinogens and whether or not the type of lesion induced can be correlated with the ability of different cell strains to repair damage induced by particular chemical agents.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: To examine the DNA repair capacities of cell strains derived from persons with genetic diseases in which there is a high probability for cancer. Specifically, to measure repair synthesis, cell survival, and DNA strand breakage and rejoining in these cells after treatment with a variety of chemical carcinogens.

Methods Employed: A simple methodology utilizing benzoylated naphthoylated DEAE cellulose chromatography (BND-cellulose) for the rapid quantitative estimation of excision repair and the rigorous separation of repair synthesis from semi-conservative replicative synthesis was employed. In the presence of hydroxyurea, an inhibitor of semi-conservative DNA synthesis, the progression of DNA chains is slowed and isotope incorporated by residual replicative synthesis will remain in the growing point region. Because the growing point contains single-stranded regions, DNA containing the newly incorporated isotope will adhere to the column and require formamide for elution. Isotope incorporated by repair synthesis into the bulk of the DNA (double-stranded) will be eluted with native DNA in the 1.0 M NaCl eluate. After corrections for thymidine pool size are made, the specific repair activity induced by any treatment can be calculated from the radioactivity and absorbancy at 260 nm of the 1 M NaCl eluate. By utilizing this method, agents which induce large-patch (UV-like) repair synthesis can easily be distinguished from those inducing small-patch (X-ray-like) repair synthesis.

The effects of carcinogen treatment on cell survival can be determined by measuring the ability of cells to form post-treatment colonies. Using colony forming ability in conjunction with the BND-cellulose technique, repair synthesis can be correlated with cell survival after carcinogen treatment.

BND-cellulose chromatography can also be used to study carcinogen-induced DNA strand scission in different cell strains. Localized single-stranded regions are formed in DNA during normal semiconservative synthesis. Chemical carcinogen treatment inhibits DNA synthesis, resulting in an accumulation of partially single-stranded DNA. Also, carcinogens can directly break DNA resulting in single-stranded breaks. Because of this single-strandedness, the amount of strand-breakage and rejoining in both parental and newly synthesized DNA can be determined using BND-cellulose columns.

Major Findings: The BND-cellulose method for quantitating DNA repair synthesis was used to measure repair synthesis induced in log phase fibroblast cell strains derived from five normal individuals and six patients with the autosomal recessive disease, Ataxia telangiectasia (AT) after treatment with ultraviolet light (UV), methyl methanesulfonate (MMS), and N-methyl-N -nitro-N-nitrosoguanidine (MNNG). All AT strains tested had normal levels of DNA repair synthesis after treatment with MMS or UV. UV induced six times more repair synthesis than did MMS, thus showing the typical pattern of repair induced by large patch and short patch repair inducing agents. Relative intercellular thymidine pools were measured to allow for accurate calculation

of the specific repair activity. When treated with MNNG, normal cells showed levels of repair synthesis intermediate between those observed after UV and MMS treatment. Four AT cell strains exhibited only 21-45% of the DNA repair synthesis shown by normal human cell strains after MNNG treatment. Two AT strains showed normal levels of MNNG-induced repair. A similar pattern of DNA repair has been observed for the same strains after ionizing radiation treatment. Four AT cell strains tested, showing either reduced or approximately normal levels of MNNG-induced DNA repair synthesis, showed decreased survival after MNNG treatment as assayed by colony forming ability. Previously, the only defect observed in the AT repair response was sensitivity to ionizing radiations. Semiconservative DNA synthesis immediately after MNNG-treatment was diminished equally in all normal and AT strains tested.

Repair synthesis following treatment with chemical carcinogens is also being studied in cell strains derived from patients with other genetic disorders with an enhanced frequency of malignancy: Fanconi's anemia, Down's syndrome, and Cockayne's syndrome. Some preliminary experiments with cell strains derived from Cockayne's syndrome patients indicate normal repair synthesis following UV and MMS and a possible diminished repair and survival after MNNG.

Experiments are beginning to further characterize the cellular response of AT cells to MNNG-treatment. It has previously been shown that excision repair induced by MNNG occurs to a greater extent in growing point regions of the DNA of a human lymphoblastoid cell line than in the bulk of the DNA, even though the overall amount of MNNG-induced alkylation is the same for replicating and non-replicating regions of the DNA. Because of the enhanced sensitivity of AT cells to MNNG, it is of interest to examine whether or not these cell strains exhibit a similar differential growing point-repair response. Also, it is of interest to determine what MNNG-induced damage is responsible for the increased sensitivity of AT cell strains. Thus, BND-cellulose chromatography is being utilized to examine the effects of MNNG induced strand-breakage in these strains in both parental and newly synthesized DNA.

Significance to Biomedical Research and the Program of the Institute: Present evidence indicates that chemical carcinogens exert their carcinogenic and mutagenic effects by alterations in cellular DNA. The manner in which cells recognize and eliminate DNA damage is important in assessing an agent's potential for cancer induction. A systematic study of genetic diseases with both a high incidence of cancer and a diminished capacity for DNA repair can lead to useful information concerning the etiology of cancer and the role of DNA repair protecting the organism from potential carcinogenic damage.

Proposed Course of Project: To pursue the goals outlined in Objectives and to publish these results.

Publications

Day, Rufus S., III, Scudiero, Dominic, A., and DiMattina, Michael. "Excision repair by human fibroblasts of DNA damaged by r-f, t-8,-dihydroxy-t-9,10-oxy-7, 8,9,10 tetrahydrobenzo(a)pyrene" Mutation Research, In press.

Day, Rufus S., III, Scudiero, Dominic A., and DiMattina, Michael "Repair of DNA damaged by benzo(a)pyrene diol-epoxide I" in Polycyclic Hydrocarbons and Cancer: Environment, Chemistry, Molecular and Cell Biology, H.V. Gelboin, and P.O.P. Ts'o, eds. Academic Press, New York, In press.

Scudiero, Dominic A. "Repair Deficiency in N-methyl-N'-nitro-N-nitrosoguanidine treated Ataxia telangiectasia fibroblasts" in DNA Repair Mechanisms (ICN-UCLA Symposia on Molecular and Cellular Biology, Vol. 9) P.C. Hanawalt, E.C. Friedberg, and C.F. Fox, eds. Academic Press, New York, In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-5001-01-CH

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

The Role of DNA Repair Damage and Its Repair Mechanisms in *In Vitro* Chemical Transformation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. Lo	Senior Staff Fellow	CH	NCI
OTHER:	T. Kakunaga	Head, Cell Growth Regulation Section	CH	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Chemistry Branch, Carcinogenesis Program

SECTION

Cell Growth Regulation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the biochemical mechanisms of *in vitro* transformation in variants subcloned from BALB 3T3 by chemical carcinogens. Present interests were on the comparison of benzo(a)pyrene (BP) metabolism and its binding to cellular DNA. Experimental results indicated that there is no major difference in these respects between the variants. Only one type of carcinogen-nucleoside adduct was found (i.e. Benzo(a)pyrene Diol epoxide I-^{2}N-guanosine). The repair of damaged DNA by variants will be studied.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-5001-01-CH

Project Description

Objectives: To study the biochemical mechanism of *in vitro* transformation in variants subcloned from BALB 3T3, for the purpose of understanding their variabilities in response to chemical carcinogens.

Methods Employed: 1) Variants of BALB 3T3 clone 1 were grown and treated with [^{3}H] Benzo(a)pyrene (BP). 2) Metabolic activation of BP in variants were measured by their induced aryl hydrocarbon hydroxylase activities (AHH). 3) Metabolite analysis were performed on high-pressure liquid chromatography (HPLC). 4) DNA was isolated from variants treated with [^{3}H]BP. 5) Carcinogen (BP) nucleoside adducts were obtained by enzymatic digestion of [^{3}H] BP-DNA and followed by chromatography on Sephadex LH20 columns. Adducts were finally identified by HPLC.

Major Findings: 1) Variants showed (a) same level of inducible AHH activity, and (b) similar metabolite patterns, suggesting these cells possess the same level of metabolic activation of carcinogens. 2) Extents of carcinogen bound to DNA in these cells are at the level of 20p mole of [^{3}H]BP/ug of DNA. 3) Only one carcinogen-nucleoside adduct was found in these variant cells. HPLC analysis identified the adduct was Benzo(a)pyrene diol epoxide-guanosine.

Significance to Biomedical Research and the Program of the Institute: The results from this project will provide information about the mechanism of cell transformation, in particular the process of initiation of transformation by chemical carcinogens. The information obtained will be useful for the development of the system for assay of environmental carcinogens.

Proposed Course of Project: To pursue the goals as outlined in Objectives and to publish any results so obtained.

Publications

Ikenaga, M. and Kakunaga, T.: Excision of 4-nitroquinoline-1-oxide damage and transformation in mouse cells. *Cancer Res.* *37*: 3672-3678, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP-05003-01 CH

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)
Microsome Catalyzed Covalent Binding of (-)r-7,t-8-dihydroxy-7,8-dihydrobenzo(a)pyrene to DNA.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Belvedere	Visiting Scientist	CH NCI
OTHER:	H. V. Gelboin	Chief, Chemistry Branch	CH NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Chemistry Branch, Carcinogenesis Research Program

SECTION
Molecular Carcinogenesis

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the in vitro microsome catalyzed covalent binding of (-)r-7,t-8-dihydroxy-7,8-dihydrobenzo(a)pyrene to DNA and the effect of this binding on various chemical carcinogenesis inhibitors and binding modifiers.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: (1) To develop an assay to study the in vitro microsome catalyzed covalent binding of (-)r-7,t-8-dihydroxy-7,8-dihydrobenzo(a)pyrene to DNA. (2) To study the effect on the DNA binding of 7,8-benzoflavone (AHH inhibitor), butylated hydroxytoluene and L-ascorbic acid (chemical carcinogenesis inhibitors), bovine serum albumin and glutathione.

Methods Employed: Spectrophotometry and liquid scintillation counting. Microsomes from control, phenobarbital and 3-methylcholanthrene treated rats were prepared according to previously published procedures.

Major Findings: DNA binding was inhibited by 7,8-benzoflavone and butylated hydroxytoluene, the extent of inhibition depended on the inhibitor and the type of microsomes. L-ascorbic acid had no effect on DNA binding. DNA binding was also inhibited in the presence of glutathione and glutathione transferase and stimulated in the presence of glutathione alone. An increase in the binding was also observed when albumin was added to the incubation mixture; this increase might be related to an increase in the half-life of the diol epoxides I and II that are the metabolic products of (-)r-7,t-8-dihydroxy-7,8-dihydrobenzo(a)pyrene.

Significance to Biomedical Research and the Program of the Institute: To identify substances that decrease binding to DNA by acting on the metabolic production or the stability of the reactive species might help to prevent one of the early steps in the initiation of benzo(a)pyrene carcinogenesis.

Proposed Course of Project: To extend this investigation to other chemical carcinogenesis inhibitors and to find other agents that could possibly decrease the half-life of the diol epoxides.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-5011-01-CH

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Studies on the Co-Mutagenic and Co-Carcinogenic Effects of Heavy Metal Ions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Kakefuda	Medical Officer	CH NCI
OTHER:	T. M. Bak	Microbiologist	CH NCI
	M. Kimura	National Institute of Industrial Health	Tokyo, Japan

COOPERATING UNITS (if any)

T. Sugimura, Director, National Cancer Center Tokyo, Japan

LAB/BRANCH
Chemistry Branch, Carcinogenesis Program

SECTION
Nucleic Acids Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

(1) The covalent binding of benzo(a)pyrene metabolite, trans 7,8-diol and 9,10-epoxide B(a)P (DE1) to DNA in vitro was significantly increased in the presence of Pb++, Hg++, Zn++ and Na+ but not with Ca++ and Mg++. (2) DE1 binding to the phosphate groups of DNA increased (54%) in the presence of La+++ while the majority of the binding occurred with guanine. (3) The mutagenesis test with Salmonella typhimurium showed about a 2 to 3 fold increase in His+ mutants when La+++, Mn++, Pb++ and Ni+++ were added to DE1. These metal ions tested have little, if any, mutagenic potential by themselves. Some metals such as Hg++ and Zn++ induced a low molecular weight protein (metallothionein) in African green monkey cell lines. The induction is transcriptionally regulated and Ca++ dependent. The role of the inducible protein in cellular defense against toxicity and muta-carcinogenesis is under investigation.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: To observe the co-mutagenic and co-carcinogenic effects of heavy metal ions which are commonly present in the environment together with known muta-carcinogens such as B(a)P. (2) To observe the molecular interaction among muta-carcinogens, DNA and heavy metal ions. (3). To observe the cellular defense mechanism against toxic and co-muta-carcinogenic heavy metal ions.

Methods Employed: (A) DE1 and closed circular DNA of plasmid PMB 322 or SV 40 virus were incubated in the presence or absence of metal ions. The DNA washed with organic solvents was then subjected to alkaline treatment, heat, and stepwise digestion with single stranded endonuclease S1 followed by pancreatic DNase. The hydrolysates thus obtained were fractionated in Sephadex LH-20 column and analyzed with TLC, high pressure chromatography and NMR. (B) Different concentrations of metal ions were added to S. typhimurium mutation assay system in addition to or absence of DE1. The number of revertant colonies was counted. (C) Established or primary cultures of cells derived from different organs and species were exposed for several hours to different radioactive heavy metal ions. The cell homogenate was centrifuged at 800xg for 10 min and the supernatant was charged onto a Sephadex G-75. The specific low molecular weight protein (metallothionein MT) was further purified and analyzed for amino acid sequencing. The mechanism of the protein induction was studied using inhibitory drugs of RNA, DNA and protein synthesis. The dependency of induction was studied by modifying the chemical and nutritional constituents of the culture media, variety of cell lines with different stages of aging, transformed cells and their temperature sensitive mutants.

Major Findings: DE1 binding to DNA in vitro depends on the concentration of DE1 in the reaction mixture which contains 36% methanol and Tris-HCl buffer pH 7.8. DE1 bound to guanine 20 times more than adenine. When La+++ was added (10^{-5}M) the total binding increased by 54% and single stranded endonuclease S1 sensitive sites increased by 10-fold over the control sample. S1 hydrolysate contained 80% of DE1-phosphate adducts. This indicated that the small amount of La+++ enhances the binding of DE1 to DNA particularly with the phosphate groups. Pb++, Hg++, Zn++ showed similar but lesser degrees of enhancement. When heavy metal ions described above were tested in S. typhimurium (TA100 and TA98 strains), no significant number of His+ revertant colonies were found. However, when these ions were added to a small amount of DE1 the mutation rate increased by 2 to 3-fold. Ca++, Mg++, Cr+++ and Cd++ ions by which the binding of DE1 to DNA was not enhanced showed no co-mutagenic effect. Hg++ was too toxic to this test system. A variety of animals including mammals and avian species are capable of producing MT which binds specifically to Cd and Zn, Cu and Hg administered to the animals. Similar proteins were isolated from kidney and liver of patients who suffer chronic intoxication of these metals. African green monkey kidney cell line and primary culture can induce MT which is identical in chemical structure to

that produced in the corresponding animal. This provided an excellent system to study the mechanism of induction. We found that a number of other cells such as fibroblasts can induce MT although MT was not found in animals administered with Cd. The induction is transcriptionally controlled as evidenced by the blockage of induction by actinomycin D. The induction is highly dependent on the calcium concentration in the culture media. Aging of the chick embryo cells (16 sub-culture) diminished the rate or induction. Transformation of the chick embryo cells by Rous sarcoma virus reactivated the inducibility of MT.

Significance to Biomedical Research and the Program of the Institute: Environmental pollutants usually consisted of several different chemical agents, such as carcinogenic hydrocarbons and non or weak carcinogens like heavy metals. The present study clearly indicates that the mutagenecity of B(a)P metabolite DE1 was enhanced significantly by the presence of heavy metals. Interaction of DE1 to DNA was also enhanced by the presence of such metal ions which can increase the mutagenecity of *S. typhimurium*. The combined model systems we established to analyze the biological and molecular consequences which may occur in the living system will be highly useful for our understanding of complex environmental carcinogenesis problems.

Proposed Course of the Project: The interaction of metal ions, ultimate active forms of carcinogens and DNA will be studied further employing advanced sensitive and accurate methods such as the purification of reaction products with high pressure chromatograhy and analysis with NMR. The cellular defense mechanism by the induction of MT against toxic and muta-carcinogenic metals will be studied with already established cell culture system. Information obtained from studies at the molecular and cellular levels will be assembled and made available for environmental hygiene against cancer.

Publication

Kakefuda, T., Yamamoto, H. and Bak, T.: Enhancement of mutagenecity of benzo(a)pyrene metabolite by metal ions. Mutation Res. (1978) In Press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-5012-01-CH

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Effects of Carcinogens and Mutagens on the Structure and Replication of DNA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Kakefuda	Medical Officer	CH	NCI
OTHER:	H. Mizusawa	Visiting Fellow	CH	NCI
	T. M. Bak	Biologist	CH	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Chemistry Branch, Carcinogenesis Program

SECTION
Nucleic Acids Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The topic of the present study is the establishment of a highly sensitive method to detect the structural modification of DNA caused by the bindings of carcinogens. Electron microscopy, gel electrophoresis and stepwise digestion with specific nucleases of double stranded circular DNA provided evidence that the binding of carcinogens to different DNA bases and phosphate groups resulted in different types of modifications. (2) The effect of the binding and modification of DNA on its replication could be studied with already established in vitro assay systems using plasmid Col El DNA and E. coli cell extract. Factors involved in the replication of normal and carcinogen damaged DNA and its repair are under investigation.

PHS-6040

Project Description

Objectives: (1) To observe the biophysical and biochemical effects of carcinogens binding to DNA. (2) To observe the consequences of DNA replication which may or may not be affected by the binding of chemical carcinogens to DNA bases and phosphate groups.

Methods Employed: (A) Benzo(a)pyrene metabolite, 7,8-diol and 9,10-epoxide B(a)P (DE1) and closed circular DNA of plasmid PBR-322 or SV 40 virus were incubated at 37°C for 30 minutes and washed with methanol, benzene and ether to remove non-covalent binding. The binding and structural modification of DNA were analyzed in agarose gel electrophoresis, electron microscopy, alkali treatment and sensitivity to endonuclease S1 and pancreatic DNase. Hydrolyzed adduct was analyzed in Sephadex LH-20 column, TLC, high pressure liquid chromatography and NMR. Synthetic double stranded polymers reacted with DE1 were also subjected for similar study. (B) Exponencially grown E. coli GM 31 was treated with lysozyme and 5% Brij 58 followed by centrifugation 25,000 rpm (SW 50.1) for 20 minutes. The resulting clear lysate supplemented with ribo- and deoxy-nucleoside triphosphates and others was used for incubation with purified PBR-322 plasmid DNA. The nasent DNA was labeled with ^{32}P-TMP or ^{32}P-ATP and/or BudR and analyzed by centrifugation in either Eth-Br-CsCl or sucrose gradients. The initiation, elongation and segregation of DNA was observed by agarose gel electrophoresis or by electron microscopy after treatment of the DNA with endonuclease Eco R. (C) Active ultimate forms of chemical carcinogens and mutagens such as DE1 were incubated with purified PBR-322 DNA as described in (A) and tested for DNA replication *in vitro* as described in (B). (D) Diluted heavy metal ions such as La, Hg, Pb, Ni, Se, Mn, etc., were added to the reaction mixture of DNA and carcinogens. Effects of these ions on the binding, modification of DNA and its modification were analyzed and compared with the metal ion free experiments.

Major Findings: Under the experimental conditions employed, the number of DE1 molecules bound to each Col E1 DNA which consisted of 6000 base pairs depended on the concentration of DE1 in the incubation media. Agarose gel electrophoresis and electron microscopic observation of the DE1 treated DNA revealed a marked conformational change (unwinding of the superhelix and breaks of strand(s)). A small fraction of the DE1 binding sites became sensitive to single endonuclease S1. TLC analysis of S1 hydrolysate showed that the S1 sensitive sites were formed by binding to adenine. The numerical majority of the binding to guanine did not cause any steric hindrance in the DNA double helix. An experiment in which double stranded synthetic polymers, poly(dA-dT).poly(dA-dT) and poly(dG-dC).poly(dG-dC), reacted with DE1 showed only the former induced S1 hydrolyzable strand separation, supporting the concept that the binding to adenine causes local denaturation. Plasmid Col E1 DNA can replicate in crude cell extract of E. coli *in vitro*. The mode of replication and products were found to be essentially the same as that of natural replication in the cell. The replication is dependent on the synthesis of primer RNA

at the initiation site. The initiation is sensitive to rifampcin and actinomycin D, while the elongation of the polynucleotide chain was not. When PMB322 DNA (derived from Col E1) was modified by a smallnumber of bindings of DE1, the initial verocity of DNA replication in the in vitro system described above was not changed in the first 30 minutes while the incorporation of dTTP increased significantly suggesting some repair synthesis was involved.

Significance to Biomedical Research and the Program of the Institute: It has been a generally accepted concept that the binding of ultimate forms of carcinogens and mutagens to DNA is a most important step in the malignant transformation of the cells. The highly sensitive and accurate system we established to analyze the binding and modification of DNA provided important information of the interaction of chemicals with the genetic machinery of the cells. The in vitro DNA replication system is also useful to investigate the effect of carcinogens on the fidelity of synthesis, damage and subsequent repair of DNA under simplified and precisely controlled experimental conditions.

Proposed Course of the Project: The DE1 DNA adducts will be further purified by high pressure chromatography and analyzed by NMR to determine the molecular structure. The source of the cell extract will be chosen from a variety of mutant strains of E. coli in order to determine the specific gene products required for replication and repair of carcinogen damaged DNA. The nature of DNA damage and subsequent repair in terms of replication process will be studied.

Publications

Kakefuda, T. and Yamamoto, H.: Modification of DNA by benzo(a)pyrene metabolite diol-epoxide 1. In Gelboin, H.V., and Ts'o, P.O.P. (Eds.) Polycyclic Hydrocarbons and Cancer: Chemistry, Molecular Biology and Environment. Academic Press, New York and London, 1978 (In Press).

Kakefuda, T., and Yamamoto, H.: Modification of DNA by the benzo(a)pyrene metabolite diol-epoxide r-7,t-8-dihydroxy-t-9,10-oxy-7,8,9,10-tetrahydrobenzo(a) pyrene. Proc. Nat. Acad. Sci. USA 75: 415-419, 1977.

Kakefuda, T., Lovinger, G.G., Gilden, R.V., and Hatanaka, H.: Electron microscopic studies of circular DNA in mouse embryo fibroblasts infected by Rauscher Leukemia virus. J. Virol. 21: 792-795, 1977.

SUMMARY REPORT

EXPERIMENTAL PATHOLOGY BRANCH

October 1, 1977 through September 30, 1978

"Plans, develops and implements a coordinated research program involving: (1) study of experimental tumor pathology; (2) development and characterization of experimental models of major forms of human cancer using in vivo and in vitro carcinogenesis methods; (3) development of techniques for the experimental study of the pathogenesis of cancer in human tissues; (4) morphological and biochemical studies on chemical carcinogenesis and its inhibition in selected animal tissues; and (5) research on the etiology and pathogenesis of cancer related to perinatal exposures."

The Branch consists of the Office of the Chief and four Sections: "Differentiation Control", "Human Tissue Studies", "In Vitro Pathogenesis", and "Perinatal Carcinogenesis". Investigators in different sections of this Branch collaborate in their studies on mechanisms and models of carcinogenesis.

Personnel formerly in the Tumor Pathology Section of this Branch was administratively reassigned from this Branch to the Carcinogenesis Testing Program. This transfer, completed early in this fiscal year, has curtailed the activities of this Branch in the area of tumor morphology and histogenesis.

The main focus of investigations in the Branch is on the study of the pathogenesis of neoplastic disease using morphological and biochemical approaches at all levels of biological organization, including human tissues, animal models, organ and cell cultures as well as molecular interactions.

Particular emphasis is given to carcinogenesis studies with epithelial cells, using both animal and human tissue sources and in vitro culture methods as well as whole animal experiments.

There is a fundamental need to relate the process of carcinogenesis to the specific charactertistics of the tissues from which the induced tumors originate. Chemical carcinogenesis is the result of a chemical-biological interaction resulting in a variety of pathologic responses characteristic of the tissues and cells of origin and of the conditions of exposure. Cancer in man is similarly characterized by a wide variety of pathologic response patterns.

Numerous animal models for major forms of human cancer, especially those of epithelial origin, were developed in recent years. These models became the necessary tools for studying the pathogenesis of major types of cancer. Such studies have rapidly advanced by combining both morphological and biochemical methods, focused on the target tissues.

Most of the current knowledge of carcinogenesis comes from studies in animals, and a great need exists to correlate animal with human pathology. This is a crucial problem in the interpretation of research findings in terms of human cancer pathogenesis and also in the assessment of carcinogenic risks for humans on the basis of experimental animal studies.

An effective approach to this problem has been established by developing methods for the experimental study of the action of carcinogens directly on human target tissues. This was accomplished - ethically and effectively - using organ and cell cultures of human tissues, particularly from the epithelial organs in which major types of human cancers are induced such as bronchus, colon, esophagus, pancreatic duct, and epidermis - for which culture methods were developed.

The metabolism of several carcinogens, their localization and their binding to cellular macromolecules have been identified in various human target tissues and cells. The DNA adducts formed by several carcinogens in human tissues were found to be the same that were previously identified in animal tissues. The wide interindividual variation in the level of carcinogen binding to tissues from different individuals has been confirmed in several studies using different carcinogens. The significance of this parameter as an indicator of susceptibility is being further investigated.

A human tissue mediated mammalian cell mutagenesis system was developed and used to demonstrate that human cells and tissues can metabolically activate procarcinogens to a reactive mutagenic form. Marked interindividual variations were again found in measuring this parameter.

Mouse epidermal cell culture methods previously established in this Branch have been further developed for use in the study of the mechanisms of neoplastic transformation and of tumor promotion in these cells, for which there is a very extensive counterpart of in vivo studies on carcinogenesis and promotion. The action of carcinogens, promoting agents and inhibitors has been investigated at the morphological and biochemical level, particularly in newly established mouse epidermal cell strains having different levels of growth ability and tumorigenicity. Markers of differentiation in epidermal cells were identified.

Organ and cell culture model systems were developed for various tissues related to different in vivo animal models of carcinogenesis.

Transplacental carcinogenesis studies in primates (Erythrocebus patas) using the carcinogen ethylnitrosourea (ENU) showed that the first one-third of pregnancy is the most susceptible period for the induction of neoplasia in the offspring. The adult females given ENU during pregnancy developed a high incidence of metastatic choriocarcinoma, providing the first experimental model for the systemic induction of this neoplasm.

Species, strain and organ specificities were investigated and metabolic activation pathways were studied for several procarcinogens and their reactive metabolites.

A highly sensitive and specific radioimmunoassay was established for the DNA-carcinogen adduct of acetylaminofluorene. Additional radioimmunoassays are being developed against DNA adducts of different environmental carcinogens: they should be most valuable in identifying and localizing target tissue exposures both in the experimental animals and in the human situation.

Mechanisms of DNA damage and repair by various carcinogens have been further defined in several of the biological model systems used in the Branch.

Cell surface properties characteristic of the transformed state, i.e. low adhesiveness and high saturation density, were reversed in cultured transformed cells by exposure to retinoids, suggesting a role of retinoids in controlling the expression of the transformed state. Further advances were made in the biochemical mechanisms of action of retinoids in the biosynthesis of membrane glycolipids and glycoproteins.

Quantitative studies of dose-response relationships and synergistic effects of carcinogens were developed using mutagensis and cell transformation models.

Criteria for the evaluation of environmental carcinogens were further reviewed and developed on the basis of the state of scientific knowledge in this field.

Office of the Branch Chief - The following activities were contributed by staff members of this Office.

In vitro systems, including the Salmonella histidine revertant mutation test (Ames) and the Balb/c 3T3 mouse fibroblast neoplastic transformation assay in culture (Kakunaga) were set up for dose-response studies on the effects of carcinogens. A comprehensive literature data analysis was undertaken to study dose-response relationships in carcinogenesis and to define criteria for evaluation of carcinogen potency.

Definitions and classifications for toxic and carcinogenic effects were outlined. Criteria for the evaluation of environmental carcinogens were further developed and scientific advice was provided to several agencies of the Federal Government and to other organizations, nationally and internationally, on the policies for environmental cancer prevention.

Differentiation Control Section - *"(1) Studies molecular processes involved in the control of cell differentiation with emphasis on maintenance of the normal phenotype and induction of new phenotypic expression by dietary, hormonal carcinogenic compounds, in the whole animal and in cultured cells; (2) investigates the biochemical mode of action whereby selected compounds inhibit or prevent the development of epithelial cancer; and (3) studies qualitative and quantitative alterations in biosynthetic and structural properties of membrane systems from normal, transforming and transformed tissues in culture and in vivo."*

Recent studies have demonstrated that derivatives of vitamin A (retinoids) are effective preventive agents in chemical carcinogenesis and alter growth properties of transformed cells. It was proposed to investigate the possibility that some of these actions of retinoids may be mediated by their biochemical effects on membrane systems and in particular through their involvement in the biosynthesis of membrane glycolipids and glycoproteins.

Spontaneously or chemically transformed epithelial and mesenchymal cells were found to reach a lower saturation density when cultured in the presence of retinoic acid and retinol. This effect was reversible upon culturing in

unsupplemented medium. Retinoids did not influence the number of cell divisions, since cultures seeded at different cell densities reached the same final density. Cell death was not enhanced by the retinoid.

A newly discovered property of retinoids is their activity in increasing the adhesion of spontaneously transformed mouse fibroblasts (Balb/c 3T12-3 cells): 95% or more of the control 3T12-3 cells can be lifted from the tissue culture surface in an EDTA adhesion assay; retinoid treatment at 1×10^{-6} M enhances the adhesive properties of transformed cells, so that only about 5% of them are lifted in this assay.

Retinoid-treated 3T12 cells reacquire their characteristic poor adhesiveness, after culturing for two days in unsupplemented medium. Thus this effect is reversible and appears to involve the cell surface. Retinol, retinyl phosphate and retinoic acid, all coumpounds with vitamin A activity, are active in lowering saturation density and increasing adhesion of 3T12-3 cells. Perhydroretinol, a compound without vitamin A activity, is inactive in this system.

These studies suggest that retinoic acid alters the surface of transformed cells in the direction of a normalized phenotype and warrant further investigation of their biochemical mode of action in this system.

Human Tissue Studies Section - *"(1) Conducts carcinogenesis studies on the pathogenesis of human cancers, especially carcinomas, using tissues from human target organs and from experimental animals, by organ culture and related methods and by correlation with clinical studies; and (2) conducts ultrastructural studies, advises and assists Carcinogenesis Program staff on electron microscopic and quantitative autoradiography techniques."*

Model systems for human carcinogenesis: Carcinogenesis researchers have striven for decades to interpret data obtained from animal studies in terms of their applicability to humans. The carcinogenic hazard of environmental chemicals has been determined primarily from studies with experimental animals and from retrospective epidemiological investigations. An experimental approach to the direct study of carcinogenesis in human tissues and cells was recently developed to assess the: a) mechanisms of carcinogenesis in human cells; b) variation of carcinogenic susceptibility among individuals; and c) validity of the extrapolation of carcinogenesis data from experimental animals to the human situation. Studies of carcinogenesis can now be conducted directly in human target tissues. In parallel with studies of human tissues, an appropriate animal model for each target site is being investigated. This approach provides a link between carcinogenesis studies using experimental animals and carcinogenesis in humans.

Model systems have been developed for human bronchus, colon, esophagus, peripheral lung, and pancreatic duct. These model systems have three major facets: 1) collection of viable human tissues; 2) in vitro maintenance of the tissues; and 3) xenotransplantation of human tissues into immunodeficient animals to assess tumorigenicity of suspected malignant cells transformed in vitro. Human tissues can be exposed to carcinogens and to anticarcinogens while in culture and/or as a xenograft. Explant cultures of human tissues

have the advantage of retaining normal cellular differentiation and the 3-dimensional intercellular relationships. Monolayer epithelial cell cultures can be initiated from explants and used for carcinogenesis studies.

The long-term organ culture of human bronchus and pancreatic duct was previously reported. During this fiscal year major accomplishments in the organ culture of human colon and peripheral lung have been made. These human tissues have been cultured as explants in a chemically-defined medium for periods of at least 20 days. The long-term organ culture of human colon has provided an opportunity to study the metabolism of chemical carcinogens for the first time directly in this important target tissue.

The metabolism of chemical carcinogens was studied in human tissues. The in vitro maintenance of human bronchus, esophagus, peripheral lung, colon, and pancreatic duct in a controlled experimental setting provides an excellent system to study the metabolism of chemical carcinogens including those found in tobacco smoke and in the environment.

Cultured human bronchi and colon were found to activate metabolically the following procarcinogens into forms that bind to cellular macromolecules including DNA: The polynuclear aromatic hydrocarbons 7,12-dimethylbenz[a]-anthracene, 3-methylcholanthrene, benzo[a]pyrene (BP) and dibenz[a,h]anthracene; the N-nitrosamines dimethylnitrosamine (DMN), diethylnitrosamine (DEN), N-nitrosopiperidine (NPd), N-nitrosopyrrolidine (NPy) and N,N'-dinitrosopiperazine (DNP); a hydrazine, 1,2-dimethylhydrazine (1,2-DMH); and a mycotoxin, aflatoxin B_1 (AFB_1).

In studies on polynuclear aromatic hydrocarbons the pathway of BP metabolism leading to its binding to DNA in cultured human bronchial and colonic mucosa has been defined. The major reactive metabolite is (+)-7α,8β-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (diol epoxide I) which forms a BP-DNA adduct between the 10-position of BP and the 2-amino group of guanine. An adduct between diol epoxide II and the 2-amino group of guanine was also found in colonic DNA from 3 of 5 patients, but not in human bronchus. The metabolic pathway forming the predominant BP-DNA adduct in human bronchus and colon is similar to that found in experimental animals in which BP is carcinogenic.

In addition to recently reported studies of BP metabolism in human bronchus, the metabolism of BP in cultured human colon was extensively investigated. Non-tumorous colonic tissue was collected at the time of either surgery or "immediate autopsy" from patients with or without colonic cancer. After 24 hrs in culture the explants were exposed to BP for another 24 hrs and the binding to cellular DNA and protein was measured. As mentioned above, two adducts formed between BP and DNA have been isolated. The major metabolites of BP extracted with ethylacetate/acetone from the tissue culture media were isolated by high-pressure liquid chromatography. The relative distribution of BP metabolites formed by cultured colon varied among individuals. About 10% of the metabolites remained in the water phase after extraction with ethylacetate/acetone. *Trans*-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene (7,8-diol) and quinones were the major metabolites released when the water-soluble metabolites were treated with β-glucuronidase and arylsulphatase. The binding

levels of BP to DNA in cultured colon showed a unimodal distribution (56 cases). The binding level of BP to DNA also showed approximately 5-fold variation among the different anatomical segments of the colon from the same patient; the binding level was generally highest in the ascending colon. Co-incubation of the explants with BP and either taurodeoxycholic acid or lithocholic acid increased the binding levels of BP to DNA. A significant increase in the amount of water-soluble metabolites was found when the explants were incubated with taurodeoxycholic acid; increased level of _trans_-7,8-diol in the media was also observed when explants were co-incubated with either taurodeoxycholic acid or lithocholic acid.

An individual's risk of developing cancer may depend, in part, on the balance between activation and deactivation of procarcinogens. Initial studies have focused on the formation of BP-DNA adducts as a resultant of these competing metabolic processes. The binding of BP to DNA in cultured human bronchus was measured in specimens from 100 patients. A 75-fold variation in binding levels was observed which is similar in magnitude to that found in pharmacogenetic studies of drug metabolism. Nontumorous specimens from patients with epidermoid lung cancer have significantly higher levels of BP bound to DNA, when compared with specimens from patients without lung cancer and from those with adenocarcinomas of the lung.

A 100-fold _inter_-individual variation was observed in the binding of activated BP to cellular DNA from the human colonic mucosa.

The metabolism of BP by cultured human bronchial epithelium and cultured pulmonary alveolar macrophages from the same donor was studied. After 7 days in culture explants of bronchus and pulmonary alveolar macrophages were exposed to BP, and the binding to cellular macromolecules was studied. Variation in the binding level of BP to DNA and to protein in pulmonary alveolar macrophages from different individuals was 9- and 33-fold, respectively. The metabolism of BP was further investigated in the macrophages. Both binding of BP to macromolecules and aryl hydrocarbon hydroxylase activity were dependent on the length of time the explant was in culture and on length of exposure to BP. Pretreatment of the macrophages with benz[_a_]anthracene increased both the binding level of BP and aryl hydrocarbon hydroxylase activity. When coincubated with BP, cycloheximide, 7,8-benzoflavone, or actinomycin D reduced both the level of binding and the activity of aryl hydrocarbon hydroxylase. When macrophage cultures were maintained at pO_2 greater than atmospheric air, an increase in binding level and enzyme activity was found. The major metabolites of BP formed by macrophages were 7,8-diol, (5 to 25 per cent of total metabolites) 9,10-dihydroxy-9,10-dihydrobenzo[_a_]pyrene (16 to 39 per cent) and two distinct peaks containing unidentified polar metabolites. A negative correlation between binding of BP to protein and aryl hydrocarbon hydroxylase occurred in pulmonary macrophages, but no correlation between data from bronchus and macrophages was found. In addition to releasing the proximate carcinogenic form of BP, 7,8-diol, into the extracellular space, pulmonary macrophages release ultimate mutagenic metabolites of BP. Pulmonary macrophages may play a role in the metabolic activation of chemical carcinogens during respiratory carcinogenesis, since they a) phagocytize particulates

including those carrying chemical carcinogens; b) accumulate on the respiratory epithelium at areas where bronchogenic carcinomas most frequently arise; and c) can metabolize BP to proximate and ultimate carcinogenic forms.

Metabolism of carcinogenic N-nitrosamines was studied in normal-appearing colonic and bronchial specimens. Explants of bronchi were cultured in a chemically defined medium for seven days. N-Nitrosamines [DMN, DEN, DNP, NPy, and NPd] labeled with ^{14}C were then each added to the medium at a concentration of 100 μM for 24 hrs. Measurable CO_2 was formed by bronchial explants from: 1) DMN, DEN and NPy in all four patients; 2) DNP in three of four patients; and 3) NPd in only one of four patients. In all bronchial specimens, binding of these N-nitrosamines and/or their metabolites to bronchial mucosal DNA and protein was found. Binding levels were higher to protein than to DNA. DMN methylated DNA at both O-6 and N-7 positions of guanine. Binding levels of DNP were as high as those with the two acyclic N-nitrosamines, DMN and DEN, while binding levels of NPy and NPd were lower. In cultured human colon, evidence of N-nitrosamine metabolism was obtained by measuring the formation of ^{14}C-CO_2. Variation in the ability to metabolize cyclic N-nitrosamines was observed among individuals. Under these test conditions only NPy was metabolized by all cases studied, DNP by 5 cases, and NPd only by one case. No ^{14}C-CO_2 was formed from N-nitrosonornicotine possibly due to the chemical structure (the ^{14}C-labeled atom had only one C-H bond), but non-labeled CO_2 could have been formed from other carbon-atoms in the pyrrolidine ring. Only DMN and NPy consistently formed alkylating moieties which reacted with cellular DNA in all 6 cases. DMN, DEN, NPy and DNP bound to protein; when compared to the other N-nitrosamines high binding levels of DNP to cellular protein were observed. A positive correlation was found between alkylation of DNA by DMN and CO_2 formation, while NPy did not show any correlation. The metabolism of NPy is more complex than that of DMN. Both α and β hydroxylation of NPy has been detected. In summary, human bronchus and colon can metabolize acyclic and cyclic N-nitrosamines that are found in the environment and in tobacco smoke.

Aflatoxin B_1 was activated by both cultured human bronchus and colon as measured by binding to cellular DNA and protein. The level of binding to DNA was several-fold higher in cultured human bronchus than in human colon. The major adduct formed by the interaction of aflatoxin B_1 and DNA was 2,3-dihydro-2-(N^7-guanyl)-3 hydroxyaflatoxin B_1 with the guanyl-group and hydroxy-group in trans-position. These observations add aflatoxin B_1 to the list of chemical procarcinogens metabolized by cultured human tissues.

Human tissue mediated mutagenesis models were developed. Since most chemical carcinogens are also mutagens, assays of mutagenesis are useful as screening tests for chemical carcinogens. In these assays, promutagens (procarcinogens) are activated by microsomal preparation from either experimental animals or from human tissues. However, metabolic activation of procarcinogens by microsomal preparations may not reflect metabolic activation within intact cells. A mutagenesis assay has now been developed in which promutagens are metabolically activated by explants of human target tissues, e.g., bronchus. Mutation frequency (resistance to either 8-azaguanine or ouabain) is measured in a mammalian cell line, V-79 Chinese hamster cells, which does not metabolically activate polynuclear aromatic hydrocarbons into mutagens. When human

bronchial explants are cocultivated with V-79 cells in medium containing either BP or its proximate carcinogen, 7,8-diol, a marked increase in mutation frequency is observed. This increase is dependent upon dose of either BP or 7,8-diol, duration of cocultivation, and amount of human bronchus. At equimolar concentrations, 7,8-diol was activated to at least a 5-fold more potent mutagen than its parent compound, BP. A positive correlation between binding levels of BP to bronchial DNA and frequency of ouabain-resistant mutations in V-79 cells was found. Human tissue mediated mutagenesis tests have potential for: 1) screening tests for chemical carcinogens; and 2) assays to be used in an effort to determine oncogenic susceptibility among individuals as well as among different target tissues in a single individual.

In vitro carcinogenesis studies were continued. Methods to culture human bronchial cells are being refined. Normal bronchial epithelial cells in monolayer culture can now be distinguished from cultured fibroblasts by immunological, biochemical and morphological methods. Promising markers for neoplastic human epithelial cells have also been developed. The morphological and biological effects of chemical and physical carcinogens are being investigated in cultured human tissues. Polynuclear aromatic hydrocarbons and/or amosite asbestos caused preneoplastic lesions in cultured human bronchus. Atypical bronchial epithelial cells are also released into the medium so that the cultures can be monitored by exfoliative cytological techniques. The tumorigenic potential of these lesions is being determined by xenotransplantation.

This Section has been responsible for training extramural investigators in the techniques for culturing human tissues. In addition, two NIH Conferences entitled "Methods to Culture Normal Human Cells and Tissues" and "Variation in Cancer Susceptibility" are being organized by staff of this Section.

Although this field is still in an early stage of development, it is evident that many human cells and tissues can be maintained in a controlled experimental setting and that studies of carcinogenesis mechanisms can be conducted directly in human cells.

In Vitro Pathogenesis Section - *"(1) In collaboration with other Sections of the Branch, develops in vitro models for studying the pathogenesis of major forms of human cancer utilizing both human and animal models; (2) utilizes these culture systems in biochemical, cytologic and biologic investigations to develop approaches to the prevention of cancer in humans, specifically by studying the stages in the pathogenesis of cancer to learn which are amenable to arresting or reversing the progression of the disease before it threatens life; and (3) develops procedures for evaluating the biological potentiality of lesions induced by chemical or physical carcinogens in mammalian tissues in vitro."*

This Section has directed its efforts towards developing in vitro model systems to study mechanisms of epithelial carcinogenesis, since the large majority of human cancers are derived from epithelial cells. A classic model for induction of squamous cancer, mouse epidermis, has been adapted for growth in cell culture in this laboratory and previously shown to maintain biological function in a reasonably normal manner.

During the past year a number of mechanistic studies have continued utilizing primary mouse epidermal cell cultures. In addition, differentiating cell strains have been developed which retain epithelial morphology for at least eight passages and have typical characteristics of epidermal cells by electron microscopy. These cells produce epidermoid cysts when injected into newborn syngeneic mice but do not produce tumors or grow in agar. One of these lines produces immunologically identifiable keratin. A number of other mouse epidermal cell lines of varying degrees of differentiation have been obtained after treatment of primary cultures with carcinogens. Subsequent studies have demonstrated that these lines represent a series of premalignant stages and that progression to later stages and to frank malignancy may occur spontaneously or can be induced by carcinogens and/or promoters. Epidermal culture models using hamster and human tissues are also being developed.

Markers for differentiation have been firmly established with the discovery that epidermal cells in vitro synthesize the two principal keratin proteins of mouse stratum corneum. It has also been established that one of the proteins (K_2, M.W. 60,000) is present in cells of all stages of differentiation while synthesis of the other (K_1, M.W. 68,000) leads to terminal differentiation. Antibodies have been produced against these proteins and a radioimmunoassay is being developed. Antibodies have also been produced against intact epidermal cells which cross react with mouse, hamster, and human epidermis but not fibroblasts of any species. These markers are now being utilized to study changes in differentiation products produced after treatment with carcinogens and tumor promoters. While the epidermal cell surface antigen is generally lower in transformed cells an absolute correlation of low reactivity with transformation could not be shown. On the other hand, growth in agar was found to be an excellent in vitro correlate to tumorigenicity in epidermal cell lines.

These culture systems have been utilized to study events associated with initiation of carcinogenesis by chemicals. The interaction of the carcinogen acetylaminofluorene (AAF) with epidermal cells leads to a major addition at the C8 position of guanine (dG-8-AAF) in DNA. A highly sensitive and specific radioimmunoassay has been developed to quantitate dG-8-AAF in DNA and follow its fate. After 1 hour of exposure, total C8 adducts represent 50-75% of total bound carcinogen but during the subsequent 24 hours about 40% of this is removed, presumably by DNA repair. Most, if not all, of the bound AAF is present in the deacetylated form. Since the successful demonstration of the usefulness of this technique for studies of carcinogen-DNA interactions, a number of antibodies are being developed against DNA adducts of carcinogens known to be present in the human environment. New information has been obtained concerning the interaction of the environmental carcinogen benzo[a]-pyrene (BP) with DNA. Exposure of epidermal cells to the ultimate carcinogenic species BP diol-epoxide I (anti) leads to gaps in newly replicating DNA presumably as a result of binding to the parent strand, since there is a 1:1 correlation between gaps and bound carcinogen molecules. These gaps are ultimately closed by a post-replication repair mechanism which is caffeine sensitive. Of particular importance is the finding that the less carcinogenic but equally reactive stereoisomer BP-diol epoxide II (syn) produces two gaps per

bound molecule which are also ultimately closed. This suggests that a different repair mechanism, which is less prone to error, may correct lesions induced by isomer II.

Studies utilizing mouse epidermal cells in culture continue to yield results concerning the cellular mechanisms controlling tumor promotion and progression. Utilizing inhibitors of macromolecular synthesis it has been determined that phorbol esters induce ornithine decarboxylase (ODC) activity by activating new enzyme synthesis requiring transcription and translation. In addition, the half life of ODC is prolonged by 2-3 fold indicating that these agents also inhibit enzyme degradation. Two agents which lead to a superinduction of ODC activity after phorbol esters, Actinomycin D, and fluocinolone acetonide do not further prolong enzyme half-life. Retinoids are potent inhibitors of promoter-stimulated ODC activity in vitro as they are in vivo.

The evolution of cell strains from primary epidermal cells in culture occurs at low frequency spontaneously and at high frequency after treatment with carcinogens and tumor promoters. Spontaneously derived cell strains are rarely tumorigenic and do not grow in agar. Three epidermal cell strains have been identified which convert from agar-negative to agar-positive growth after a two week exposure to phorbol esters, in analogy to late stages of tumor promotion. This transition is independent of stimulated proliferation and the efficiency of colony growth induced in agar correlates to in vivo promoting potency for a series of phorbol esters. The transition can be inhibited in vitro by retinoids just as tumor promotion is inhibited in vivo; steroids however do not appear to inhibit the induction. The transition leads to the permanent acquisition of agar-growth potential and a number of clones of varying degrees of response have been isolated.

In vivo studies to parallel in vitro models have continued during the previous year. A new model system, where human foreskins grafted to nude mice are exposed to carcinogens and/or promoters has revealed that phorbol esters produce hyperplasia in human skin grafts and papilloma formation on human skin can result. This model offers a unique opportunity to extend experimental observations to human tissues. In vivo studies on the role of proliferation at the time of tumor initiation show that proliferation shortly after carcinogen exposure is most critical and implicate a fixation of mutation or the involvement of post-replication repair in initiation.

The Section also contributed, through advice, planning and review for the collaborative program, to studies for other in vitro models of epithelial carcinogenesis representing major forms of human cancer, such as projects developing cell and organ cultures of pancreatic and prostatic epithelium and several studies to identify in vitro markers for recognizing malignant transformation in epithelial cells.

Perinatal Carcinogenesis Section - *"(1) Investigates the induction of cancer in experimental animals before birth and during infancy, with the goal of identifying factors responsible for the initiation and growth of tumor of childhood and infancy in man; (2) utilizes techniques of chemical synthesis, biochemistry, histopathology, immunology, and endocrinology to identify*

carcinogens to which both individually and in combination the fetus and neonate are particularly vulnerable, and to identify host factors which qualitatively modify their biological effects; and (3) develops measures for prevention of cancer in children or in later life as a response to conditions of high susceptibility to carcinogens during fetal life or childhood."

Carcinogenesis in primates by direct-acting and enzyme-dependent alkylating agents has been studied in the Old World monkey, Erythrocebus patas, using ethylnitrosourea (ENU) and diethylnitrosamine (DEN). Studies in collaboration with Dr. W. London and A. Palmer (NINCDS), D. Sly (Meloy Laboratories, Inc.), and G. Williams (American Health Foundation) have confirmed and extended previous observations on the relative susceptibilities of early and late fetal, juvenile, and adult primates to ENU. Eleven tumors, chiefly of hepatocellular, renal, or blood vascular origin, have now been observed in 10 offspring of patas given ENU at various times during the 165 days of pregnancy. All but 1 tumor developed in offspring first exposed to ENU before day 35 of gestation, and 8 of the 11 tumors appeared during the first year after birth. The first one-third of pregnancy is the period of maximal fetal susceptibility to this carcinogen, and the exposed offspring are at risk during infancy for tumors which resemble the pediatric tumors of man. Studies on excision of guanine O-6 alkylation products from DNA of various organs in newborn monkeys have been carried out in collaboration with Prof. P. Kleihues, Freiburg, Germany. In the monkey, as in the mouse and rat, O-6 methyl- or ethylguanine is rapidly removed from the liver but retained in the brain after treatment with ENU or its methyl homolog. As these agents readily induce brain tumors in rats, but liver tumors in mice and monkeys, it is apparent that excision of these specific alkylation products is not predictive per se of the most probable site of tumor development. Several adult females given ENU during pregnancy have died within 6 months from the time of their first injection, after either aborting their conceptus or full-term delivery of a live infant, and have been found to have widely metastatic choriocarcinoma. No other types of tumors have been observed in these animals. This observation demonstrates that pregnancy is a state of high risk for carcinogenesis, not only to the conceptus but also to the mother. It also provides the first experimental model for systemic induction of choriocarcinoma, a neoplasm not inducible in rodents by systemic exposure to carcinogens.

Studies have continued on the carcinogenic effects of synthetic α-hydroxy derivatives of dimethylnitrosamine (DMN) and diethylnitrosamine (DEN) as models of the postulated reactive and carcinogenic metabolites of alkylnitrosamines. Methyl(acetoxymethyl)nitrosamine (DMN-OAc) was previously reported to be selectively carcinogenic for the intestinal mucosa of Sprague-Dawley rats, but only when given ip. Subsequent studies in Fischer F344 and Buffalo (BUF) strain rats have revealed a marked difference in susceptibility among strains, with BUF rats highly resistant. The sex difference previously observed in SD rats is even more pronounced in the other strains. A marked shift of organotropy occurred when DMN-OAc was given by different routes, indicating that it behaves as a short-lived, direct-acting carcinogen. Both DMN-OAc and DEN-OAc are being studied for transplacental effects in rats, and early data suggest that both are carcinogenic to the fetal nervous system.

An organ culture system for metanephric rudiments from rats and mice has been developed to study renal differentiation subsequent to carcinogen exposure as a determinant of the histological patterns and biological behavior of different types of renal tumors inducible by perinatal exposure to carcinogens, which differ markedly in these two species. Conditions have been established for the growth and differentiation of kidney rudiments from 15-day fetuses in vitro for a period of 1 week. Histologic, ultrastructural, and histochemical characterization of fetal and adult normal renal epithelium from rodent and porcine sources, and of partially differentiated neoplastic cells from a human nephroblastoma, have been achieved as a basis for evaluating responsiveness of rat and mouse renal tumor cells to inducers of renal differentiation.

The role of excision repair of damaged DNA in modifying the effects of alkylating agents in various fetal organ systems has been studied comparatively in rats and mice using alkaline sucrose density gradient ultracentrifugation and thymidine-H^3-labeled primary cell cultures of liver, brain, and kidney from early fetal (15 day), late fetal (19 day), neonatal, and adult animals. Exposure to 6 mM methyl methanesulfonate (MMS) for 20 minutes caused severe DNA breakage in all tissues. Early fetal rat brain was least able to repair this damage, and was significantly less efficient than early fetal rat liver, mouse liver, or mouse brain. Studies are continuing in order to establish the relative efficiency of excision repair in different organ systems at different stages of development following exposure to MMS and other toxic and carcinogen compounds.

The molecular basis of the differential effects of ethylating vs. methylating agents has been studied in a cell-free system in which functional β-galactosidase is synthesized in vitro from bacterial lac operon DNA. Methylnitrosourea (MNU) and ethylnitrosourea (ENU) both inhibit enzyme synthesis, but MNU reacts much more rapidly with DNA and appears to act by inhibiting RNA synthesis. The relative extent of alkylation and carbamoylation of DNA by MNU is now under study. The ultimate goal of this project is to provide a system for study of the fidelity of repair of DNA damage inflicted by different classes of chemical carcinogens.

The relevance of perinatal carcinogenesis to the programs of national and international organizations, especially in the fields of disease prevention and safety evaluation, has continued to result in numerous requests for participation in formal meetings organized to discuss the utilization of research findings in this field by regulatory agencies and toxicology groups. During the past year, formal presentations with subsequent publications have been contributed to NIEHS for the US-USSR Exchange Program in Environmental Health Sciences (Leningrad, Nov. 25-Dec. 10, 1977), the Toxicology Forum (Washington, Feb. 19-22, 1978, and Ottawa, May 18-19, 1978), the Society for Occupational Safety and Health and NIOSH (Bethesda, April 19-22, 1978), and the Environmental Protection Agency (Amherst, MA, June 4-7, 1978).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04484-02 EXP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Study of the Effect of Retinoid on the Cell Surface of Cultured Transformed Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

	Name	Title	Affiliation
PI:	Luigi M. De Luca	Research Chemist	EXP, NCI
	Sergio Adamo	Visiting Fellow	EXP, NCI
	P. V. Bhat	Visiting Fellow	EXP, NCI
	Irene Akalovsky	Chemist	EXP, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Experimental Pathology Branch

SECTION

Differentiation Control Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Spontaneously transformed mouse fibroblasts (Balb/c-3T12-3 cells) were found to reach a lower saturation density when cultured in the presence of retinol or retinoic acid. Retinoids did not influence the number of cell divisions, but only the saturation density, since cultures seeded at different cell density stopped growing when they reached a density of approximately 120,000 cells/cm^2. Retinoids also caused 3T12-3 cells to become much more adhesive to the culture dish surface and to each other: about 95% or more of the untreated cells were usually lifted from the dish surface by EDTA. Retinoid treatment enhanced the adhesion of cells so that only about 5% of them were lifted in this assay. After removal of retinoids from the culture medium, saturation density and adhesion returned to control values within 48 hours.

PHS-6040
(Rev. 10-76)

Objectives: Balb/3T12-3 mouse fibroblasts were cultured in T25 flasks in 3 ml of medium. Dulbecco's Modified Eagle Medium was supplemented with 10% calf serum 25 mM Hepes, pH 7.3 and 50 μg/ml gentamicin. The medium was changed every day. Trypsinizations (both for cell counting and passage) were performed as follows: the trypsinization solution contained 2.5 g/l trypsin and 0.2 g/l EDTA in Hank's Basal Salt Solution, Ca++, and Mg++ free, pH 7.6. After removal of the culture medium the dishes were rinsed twice with 3 ml Dulbecco's Phosphate Buffered Saline (PBS), Ca++ and Mg++ free. The cells were rinsed quickly with 1.5 ml of the Trypsin-EDTA solution and incubated for 2' at room temperature with 1.5 ml of fresh trypsin-EDTA solution. After removal of the trypsin-EDTA solution, cells were incubated for 15 minutes at 37 °C and then suspended by pipetting in a suitable volume of either complete medium (for passages) or 10% serum in PBS (for cell counting). Samples of the cell suspensions obtained by trypsinization were routinely screened microscopically: more than 95% of the cells were present as single cells. Cell counting was performed using a model B Coulter Counter (Coulter Electronics, Hialeah, Florida). Trypan blue dye exclusion test was performed by incubating the cells with 1 vol. of 0.4% trypan blue in PBS and 1 vol. of complete medium. All-trans-β-retinoic acid was dissolved in dimethylsulfoxide at a concentration of 2 mg/ml or less, and the solution (freshly made every week) stored in the dark at room temperature. Retinoic acid in DMSO or DMSO alone was added to the culture medium 24 hr after plating the cells, unless otherwise stated. DMSO concentration in the culture medium was 0.5%.

The adhesion assay was performed as follows: cells cultured in T25 flasks were rinsed with 3 ml of PBS Ca++, Mg++ free; the cells were shaken at 110 rpm on a Clay Adams variable speed rotator for 4 minutes at room temperature. At the end of the incubation the detached cells were removed and these, as well as the cells still adherent to the culture surface, were trypsinized and dispersed as described, in order to obtain a single-cell suspension suitable for automatic enumeration. When indicated, the adhesion test was preceded by a rinse of the cells with diluted trypsin (0.25 g/l in PBS Ca++, Mg++ free) for about 10 seconds at room temperature (pre-trypsinization).

Major Findings: Spontaneously transformed mouse fibroblasts (3T12-3 cells) grow to reach a density of about 450,000 cells/cm^2 by the end of the second week of culture. After this time, no further increase in cell density occurs and cell death and detachment become evident. Cells cultured in the presence of retinoic acid (5-10 μg/ml) stop growing after one week of culture, when they are confluent, at a density of about 130,000-150,000 cells/cm^2.

The effect of retinoic acid on cell proliferation is apparently not due to toxicity as suggested by two observations: (a) trypan blue staining of treated and control cultures showed no difference in the percentage of stained cells in the two groups; (b) treated cells could be subcultured for several passages, in the continuous presence of retinoic acid.

Cells plated at different initial densities reach the same final density, although they undergo different numbers of cell cycles, before reaching the stationary state. This finding indicates that the final cell density is the parameter affected by retinoic acid, rather than the number of cell divisions.

It was found that trypsinization was less effective in lifting retinoic acid treated cells than in lifting control cells from the culture surface. An EDTA adhesion assay was used. A dose dependency of the effect of retinoic acid on the adhesion of 3T12 cells was found between 0.05 and 5 μg/ml. Confluent monolayers of cells were not detached as single cells but as sheets, probably containing cells of varying adhesive properties. If cells were treated with diluted trypsin prior to the adhesion test, they could be detached mostly as single cells.

A significant increase of adhesion could be measured 48 hr after the addition of retinoic acid and the maximum effect was attained at the sixth day of culture. Retinol and retinyl phosphate had the same effects as retinoic acid.

Removal of the retinoid from the culture medium restored high saturation density and poor adhesion to cells within 48 hours.

Significance to Biomedical Research and the Program of the Institute: This project was conceived in view of recent findings which implicate cell-surface glycosyl transferases, glycoproteins and glycolipids in the regulation of expression of the social behaviour of cells. Severe modification of these molecules have been shown to be associated with chemical and viral transformation. Thus it is expected that this project will contribute to the understanding of the basis for modified behaviour of transforming and transformed cells.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04485-02 EXP

PERIOD COVERED

October 1, 1977 to September 1, 1978

TITLE OF PROJECT (80 characters or less)

Mutagenesis Mediated By Human Tissues and Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	I. Hsu	Research Scientist	EXP, NCI
	C. Harris	Head, HTSS	EXP, NCI
Other:	G. Stoner	Expert	EXP, NCI
	H. Autrup	Staff Fellow	EXP, NCI

COOPERATING UNITS (if any)

University of Maryland, School of Medicine, Baltimore, MD

LAB/BRANCH

Experimental Pathology Branch

SECTION

Human Tissue Studies Section (HTSS)

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Human bronchial explants activated benzo[a]pyrene and 7,8-diol into proximate and ultimate carcinogenic metabolites that were then released into the medium containing to the cocultivated Chinese hamster V-79 cells; an increase in mutation frequency of indicator V-79 cells was observed. With the new fluorescent plus Giemsa staining method, there was also an increase in sister chromatid exchanges (SCE) in the cocultivated V-79 cells. In addition to causing a higher increase of mutation frequency mediated by human bronchus than benzo[a]pyrene, 7,8-diol showed a higher increase of SCE in cocultivated V-79 cells. 7,8-Benzoflavone, an inhibitor of the metabolism of polynuclear aromatic hydrocarbon, also inhibited bronchial explant-mediated SCE and mutation in the indicator V-79 cells. Benzo[e]pyrene, the weakly carcinogenic analog of benzo[a]pyrene, did not increase either SCE or mutation frequency in V-79 cells. In addition to bronchial explants, pulmonary alveolar macrophages and blood monocytes have the capability to mediate mutation and SCE.

PHS-6040
(Rev. 10-76)

Objectives: To study mutation of V-79 cells mediated by human tissue and cells to determine the relation between mutagenicity mediated by human cells and tissues and oncogenic susceptibility among individuals.

Methods Employed: Explant cultures of human tissues; cocultivation of V-79 cells (a Chinese hamster cell line) and human tissues in medium containing a suspected chemical carcinogen; selection of V-79 cell mutant by media containing either ouabain or 8-azaguanine; Hoechst-Giemsa chromosome stain to determine sister chromatid exchanges; isolation of carcinogen-DNA adducts in human tissues.

Major Findings: Cultured human bronchial explants activated benzo[a]pyrene (BaP) into electrophilic metabolites that bind to DNA in bronchial epithelial cells. Promutagenic and mutagenic metabolites of BaP were also released into the culture medium. An increase in mutation frequency for ouabain resistance was found in Chinese hamster V-79 cells when they were co-cultivated with bronchial explants in the presence of BaP. The proximate carcinogenic form of BaP, 7,8-diol (($\pm$)r7,t8-dihydroxy-7,8-dihydrobenzo[a]pyrene), was 5-fold more potent as a promutagen than the parent compound. Neither BaP nor 7,8-diol increased the mutation frequency in V-79 cells when they were cultured without bronchial explants. The mutation frequency was directly related to the binding levels of BaP to bronchial DNA and the concentration of either BaP or 7,8-diol in the medium.

The mutation frequencies for O^r and for SCE in V-79 cells co-cultivated with PAM and either BaP or 7,8-diol were measured. The mutation frequency was directly dependent upon the number of PAM added to the V-79 cells in medium containing either BaP or 7,8-diol. BeP, the weakly carcinogenic analog of BaP, was not mutagenic. Co-cultivation of V-79 cells with PAM but without BaP, BeP or 7,8-diol did not alter the O^r spontaneous mutation frequency of V-79 cells (less than two O^r mutants/10^6 surviving V-79 cells). An increase in concentration of either BaP or 7,8-diol enhanced O^r mutation frequencies. When compared to BaP, 7,8-diol was a more potent promutagen. PAM also metabolized BaP to 7,8-diol which was released into the culture medium.

The clearance of inhaled particulates with adsorbed chemical carcinogens may be impaired by tobacco smoke, sulfur dioxide, anticholinergic drugs, chronic bronchitis and respiratory infections. These particulates may also have a prolonged residence time in areas of squamous metaplasia because ciliary activity is interrupted in these areas. PAM phagocytize particulates and are also considered to play an important role as a host defense against inhaled foreign material. Our data suggest that PAM can also metabolize BaP to proximate and ultimate mutagens, which are then released into the extracellular space. The proximate mutagenic and carcinogenic form of BaP, 7,8-diol, can enter into cultured bronchial explants from the medium and has been shown to be more effectively metabolized and bound to DNA in the bronchial mucosa than the 4,5-diol, 9,10-diol and the parent compound, BaP. The intimate contact between PAM and bronchial epithelium during clearance by the mucociliary transport of particulates containing chemical carcinogens within PAM

suggests the possibility that PAM and bronchial epithelial may act in concert to metabolically activate chemical carcinogens during bronchogenic carcinogenesis.

Significance to Biochemical Research and the Program of the Institute: Most studies with chemical carcinogens were done in experimental animals. Extrapolation of the results to humans is a complex problem in part because of the differences in the metabolism of chemicals between species. The mutagenesis test described above will utilize human tissue from several different organs and individuals, and may provide clues in the search for host factors that influence oncogenic susceptibility.

Proposed Course of the Project: Continuation of the mutation studies mediated by human tissues and cells. Measurement and comparision of SCE, DNA repair synthesis, and mutation of V-79 cells mediated by human tissue. Development of the human tissue mediated transformation and mutation assay with human cells as the detector system. To develop a human tissue-mediated microbial mutagenesis test which uses *Salmonella* as the indicator organism.

Publications

C. Harris, I. C. Hsu, G. D. Stoner, and B. F. Trump: Human pulmonary alveolar macrophage metabolizes benzo[a]pyrene to proximate and ultimate mutagens. *Nature*, 1978, In press.

I. C. Hsu, G. D. Stoner, H. Autrup, B. F. Trump, J. K. Selkirk, and C. C. Harris: Human bronchus-mediated mutagenesis of mammalian cells by carcinogenic polynuclear aromatic hydrocarbons. *Proc. Natl. Acad. Sci.*, 1978, In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04486-02 EXP

PERIOD COVERED October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Explant and Cell Culture of Human and Bovine Pancreatic Duct

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Stoner	Expert	EXP, NCI
	C. Harris	Head, HTSS	EXP, NCI
Other:	H. Autrup	Staff Fellow	EXP, NCI
	J. M. Foidart	Research Scientist	ODBA, NIDR

COOPERATING UNITS (if any)

University of Maryland, School of Medicine, Baltimore, MD
Litton Bionetics, Inc., Kensington, MD

LAB/BRANCH
Experimental Pathology Branch

SECTION
Human Tissue Studies Section (HTSS)

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 1	PROFESSIONAL: 0.5	OTHER:

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Model systems for the study of carcinogenesis in cultured human and bovine pancreatic duct tissues have been developed. Explants of human and bovine pancreatic duct have been maintained in culture for periods of 60 and 85 days respectively. The metabolism of chemical carcinogens is being investigated in cultured human pancreatic duct. Attempts are being made to transform epithelial cells in explant tissues of human and bovine pancreatic duct and in cell cultures isolated from bovine pancreatic duct. It is anticipated that these model systems will be useful for: 1) identification of environmental carcinogens for the pancreatic duct; 2) determination of the metabolic pathways for carcinogens in the pancreatic duct; 3) identifying host factors determining susceptibility to chemically induced pancreatic cancer; and 4) evaluating new methods of prophylactic intervention in populations at high risk of developing pancreatic cancer.

PHS-6040
(Rev. 10-76)

Objectives: To investigate the effects of chemical carcinogens in human and bovine pancreatic duct. These studies involve 3 major facets: 1) obtainment of viable human and bovine ductal tissues; b) in vitro maintenance of epithelial cells in these tissues in cell and organ culture; and 3) xenotransplantation of human and bovine pancreatic duct into immunodeficient animals.

Methods Employed: Human pancreatic ductal tissues are obtained from "immediate" autopsies. Bovine pancreatic ducts are obtained from a local abatoir. The tissue is maintained in culture as explants, isolated cells, or xenotransplanted into athymic nude mice. The tissues are exposed to chemical carcinogens in vitro or as a xenograft. The morphological lesions induced in explants or in xenotransplants are examined by light and electron microscopy. The cultured cells are assayed for carcinogen-induced transformation by determining their ability to form colonies in soft agar and to produce tumors after transplantation into athymic nude mice. Binding of carcinogens to macromolecules is measured by biochemical and autoradiographic methods.

Major Findings: Normal human and bovine pancreatic ductal tissues have been maintained in explant culture for periods of 60 and 85 days, respectively. The tissues are cultured in CMRL-1066 medium supplemented with 5% heat-inactivated fetal calf serum, insulin (1 μg/ml), hydrocortisone (0.1 μg/ml) beta-retinyl acetate (0.1 μg/ml), penicillin (100 μ/ml) and streptomycin (100 μg/ml). The explants maintained good ultrastructural preservation during these periods and incorporated radioactive precursors into DNA, RNA and protein. Exposure of human and bovine pancreatic ductal explants to a single dose of N-methyl-N-nitroso-N'-nitroguanidine (MNNG) at 2.5 μg/ml resulted in epithelial cell hyperplasia with the presence of many atypical cells. Cytologically, these cells were malignant by light microscopy. By electron microscopy, the cells after 3 to 4 weeks in culture often became extremely large in diameter with high nucleo-cytoplasmic ratios, atypical nuclei, large nucleoli and a variety of cytoplasmic alterations.

Cells with an epithelial morphology have been isolated from bovine pancreatic duct and propagated continuously in culture for 26-30 subcultures (95 doublings of the cell population). By electron microscopy, the cells have junctional complexes composed of tight junctions, gap junctions and structures resembling desmosomes. They also contain inclusions with the ultrastructural features of mucus droplets. Enzyme histochemical studies have shown that the cells possess ATPase and alkaline phosphatase activity but do not possess activity for the enzyme alpha-glutamylpeptidase which is a marker for ductal epithelium in the guinea pig pancreas. Investigations of the synthesis of the various types of collagen (I, II, III, and IV) by these cells have been undertaken and compared to a line of bovine pancreatic fibroblasts. As expected, the fibroblasts produce abundant types I and III collagen and not types II or IV. The epithelial-like duct cells synthesize small amounts of presumptive type IV collagen and no types I, II or III. Attempts are being made to transform the cells with MNNG (1.0 and 0.1 μg/ml) and 4-nitroquinoline-1-oxide (4-NQO; 0.4, 0.2 and 0.1 μg/ml) given in single

or in multiple exposures. To date, these treatments have resulted in changes in cell morphology, increased growth rate, changes in karyology, and a prolongation of the life expectancy of the cells. The cultures are being monitored for the ability of the cells to grow in soft agar and to produce tumors in athymic nude mice.

Significance to Biomedical Research and the Program of the Institute: Carcinoma of the pancreas is the fourth leading cause of cancer death in the United States, and its incidence is increasing. Although the causes of pancreatic cancer are unknown, recent epidemiological studies have associated the disease with at least two variables: (a) excessive cigarette smoking, and (b) a diet high in fat and/or cholesterol. In addition, chemists and industrial workers exposed to such compounds as methylnitrosourethane, methylcholanthrene, and acetylaminofluorene have a higher incidence of pancreatic cancer than the general population. Given these results, it is important to develop experimental models for the study of chemical carcinogenesis in the human pancreas. Long-term maintenance of human pancreatic duct in explant culture has been achieved and attempts are being made to isolate and culture pancreatic epithelial cells. Bovine pancreatic duct tissues are being used as an in vitro animal model system for studies of chemical carcinogenesis. It is anticipated that methodologies developed in the bovine system might be applicable to the human tissues. These models would be useful for the study of chemical carcinogenesis in the pancreatic duct, and for the identification of host factors determining susceptibility to chemically induced pancreatic cancer.

Proposed Course of Project: Explants of human and bovine pancreatic duct have been cultured for extended periods in vitro. Biochemical studies have shown that these tissues have the ability to metabolize chemical carcinogens and experiments are underway to determine whether the epithelial cells can be transformed to malignancy with chemical carcinogens. Cells with an epithelial morphology have been isolated from bovine pancreatic duct and cultured in vitro for prolonged periods. The cells were cloned and the clones are being treated with carcinogens to determine whether they can be transformed to malignancy. Studies will be conducted to determine whether the growth of human pancreatic duct cells might be improved by examining the nutritional requirements of the cells; *e.g.*, media, serum, etc., and by culturing the cells on different substrates; *e.g.*, collagen, x-irradiated feeder layer, or gelatin sponge. After establishment of optimum growth conditions for the cells, carcinogenesis studies will be initiated.

Publications

Stoner, G. D., Harris, C. C., Bostwick, D. G., Jones, R. T., Trump, B. F., Kingsbury, E. W., Fineman, E., and Newkirk, C.: Isolation and characterization of epithelial cells from bovine pancreatic duct. *In Vitro*, 1978, In press.

Berman, J., Stoner, G. D., Dawe, C., Rice, J., and Kingsbury, E.: Histochemical demonstration of collagen fibers in ascorbic acid-treated cell cultures. In Vitro, 1978, In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04487-02 EXP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Metabolism of Chemical Carcinogens in Cultured Human Pulmonary Alveolar Macrophages

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. Autrup	Staff Fellow	EXP, NCI
	C. Harris	Head, HTSS	EXP, NCI
Other:	G. Stoner	Expert	EXP, NCI

COOPERATING UNITS (if any)

University of Maryland, School of Medicine, Baltimore MD

LAB/BRANCH
Experimental Pathology Branch

SECTION
Human Tissue Studies Section (HTSS)

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.25	OTHER:

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The metabolism of benzo[a]pyrene (BP) by human bronchus and pulmonary alveolar macrophages (PAM) from the same donor was studied to investigate whether the PAM might be useful as an indicator cell for the human bronchus. PAM were able to enzymatically convert BP into metabolites which bound to cellular macromolecules. The uptake of Fe_2O_3-BP into PAM and the release of BP and metabolites into the tissue media was determined. The effect of asbestos on this release is being investigated.

PHS-6040
(Rev. 10-76)

Objectives: To characterize the pathways of BP metabolism by cultured human PAM. To compare metabolism of BP in PAM with that of cultured human bronchus. To determine the mode of uptake of the carcinogen into the PAM and to identify various factors which could affect the release of the carcinogen into the extracellular space.

Methods Employed: Explant culture of human bronchus and cell culture of PAM; enzyme assays; isolation of cellular macromolecules; high pressure liquid chromatography.

Major Findings: The metabolism of [^{3}H]benzo[a]pyrene by cultured human bonchial epithelium and cultured pulmonary alveolar macrophages from the same donor was studied. Explants of bronchus were prepared from surgical and autopsy specimens. Human pulmonary alveolar macrophages were isolated from peripheral lung by trypsinization and by differential adhesion to plastic tissue culture dishes. After 7 days in culture the bronchus explant and the pulmonary alveolar macrophages were exposed to [^{3}H]benzo[a]pyrene, and the binding to cellular macromolecules was studied. Aryl hydrocarbon hydroxylase activity was determined by the release of tritiated water into the culture medium from metabolized [^{3}H]benzo[a]pyrene. Variation in the binding level of benzo[a]pyrene to DNA and to protein in pulmonary alveolar macrophages from different individuals showed 9- and 33-fold interindividual variation, respectively. The metabolism of benzo[a]pyrene was further investigated in the macrophages. Both binding of benzo[a]pyrene to macromolecules and aryl hydrocarbon hydroxylase activity were dependent on the length of time the explant was in culture and on length of exposure to benzo[a]pyrene. Pretreatment of the macrophages with benz[a]anthracene increased both binding level of benzo-[a]pyrene and aryl hydrocarbon hydroxylase activity. When coincubated with benzo[a]pyrene, cycloheximide, 7,8-benzoflavone, or actinomycin D reduced both the level of binding and the activity of aryl hydrocarbon hydroxylase. When macrophage cultures were maintained at pO_2 greater than atmospheric air, an increase in binding level and aryl hydrocarbon hydroxylase activity was found.

The major metabolites of benzo[a]pyrene formed by macrophages were 7,8-dihydroxy-7,8-dihydro-benzo[a]pyrene, (5 to 25 per cent of total metabolites) 9,10-dihydroxy-9,10-dihydrobenzo[a]pyrene (16 to 39 per cent) and two distinct peaks containing unidentified polar metabolites. A negative correction ($r = -0.58$; $p < 0.05$) between binding of benzo[a]pyrene to protein and aryl hydrocarbon hydroxylase exists in pulmonary macrophages, but no correlation between data from bronchus and macrophages was found.

The uptake of BP by PAM was investigated. [^{3}H]BP was either absorbed on Fe_2O_3 (336 μg BP/mg Fe_2O_3) or dissolved in dimethylsulfoxide (DMSO). When BP-Fe_2O_3 (1 mg/ml) was added to cultures of PAM (5×10^5/dish) 8%, 13% and 30% of the BP-Fe_2O_3 was phagocytized at 1, 5 and 25 hr, respectively. Following a 1 hr incubation with BP-Fe_2O_3 and then removing unphagocytized BP-Fe_2O_3, PAM released into the culture medium BP as well as metabolites of BP, e.g., its proximate carcinogenic form, trans-7,8-diol. The release of BP as well as metabolites was partly inhibited in the presence of 7,8-benzoflavone. The effect of asbestos and other fibers are currently being investigated.

Significance to Biomedical Research and the Program of the Institute: These preliminary results indicate that PAM might not be used as an indicator cell for human bronchus, the major site of lung cancer, and hence not be useful in determining an individual's risk to bronchogenic carcinoma by measuring enzyme levels in PAM. Since PAM metabolize BP to the proximate carcinogen, 7,8-diol, and release it into the extracellular space, PAM may play a role in preactivation of BP in the lung.

Proposed Course of the Project: To determine the effects of asbestos, glass-fibers, chromates and nickel particulates on the metabolism of chemical carcinogens by human pulmonary macrophages.

Publications

Autrup, H., Harris, C. C., Stoner, G. D., Selkirk, J. K., and Trump, B. F.: Metabolism of [^{3}H]benzo[a]pyrene by cultured human bronchus and pulmonary alveolar macrophages. Lab. Invest. 38: 217-223, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CP 04488-02 EXP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Metabolism of Chemical Carcinogens in Cultured Human Colon

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. Autrup	Staff Fellow	EXP, NCI
	C. Harris	Head, HTSS	EXP, NCI
Other:	None		

COOPERATING UNITS (if any)
MIT, Cambridge, MA
University of Maryland, School of Medicine, Baltimore, MD
Institute for Cancer Research, New York, NY

LAB/BRANCH
Experimental Pathology Branch

SECTION
Human Tissue Studies Section (HTSS)

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 1 | PROFESSIONAL: 0.5 | OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Explant cultures of human and colon have the ability to enzymatically convert various classes of chemical carcinogens, such as polycyclic aromatic hydrocarbons, N-nitrosamines, mycotoxins and hydrazines, into metabolites which react with cellular macromolecules, such as DNA and protein. A 100-fold interindividual variation in the binding of benzo[a]pyrene (BP) to DNA was found. The carcinogen-DNA adducts for aflatoxin B_1, BP, dimethylnitrosamine and 1,2-dimethylhydrazine have been identified. Secondary bile acids significantly enhanced the binding of BP to DNA. This effect was mediated by increased uptake of BP into the cells and by modification of the metabolic pathway of BP.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04488-02-EXP

Objectives: To compare the metabolism of chemical carcinogens in human colon to that in experimental animals. To study the effect of various co- and anti-carcinogens on the metabolism of these carcinogens.

Methods Employed: Explant cultures of human colon; enzyme assays; isolation of cellular macromolecules; high pressure liquid chromatography; autoradiography.

Major Findings: The metabolism of benzo[a]pyrene in cultured human colon has been investigated. Non-tumorous colonic tissue was collected at the time of either surgery or "immediate autopsy" from patients with or without colonic cancer. After 24 hrs in culture explants were exposed to [^{3}H]benzo-[a]pyrene for another 24 hrs and the binding to cellular DNA and protein was measured. Two adducts, formed between benzo[a]pyrene and DNA have been isolated. The major adduct (72%-100%) was formed between the 10-position of benzo[a]pyrene diol epoxide I and the 2-amino group of guanine, and the minor adduct between benzo[a]pyrene diol epoxide II and the 2-amino group of guanine. The major metabolites of benzo[a]pyrene extracted with ethylacetate/acetone from the tissue culture media were (7,10/8,9)-tetrahydroxy-7,10,8,9-tetrahydrobenzo[a]pyrene, trans-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene and a peak containing (7,9,10/8)-tetrahydroxy-7,10,8,9-tetrahydrobenzo[a]pyrene, (7/8,9)-trihydroxy-7,8-dihydrobenzo[a]pyrene and trans-9,10-dihydroxy-9,10-dihydrobenzo[a]pyrene. The relative distribution of benzo[a]pyrene metabolites formed by cultured colon varied among individuals. About 10% of the metabolites remained in the water phase after extraction with ethylacetate/acetone. Trans 7,8 dihydroxy-7,8-dihydrobenzo[a]pyrene and quinones were the major metabolites released when the water-soluble metabolites were treated with β-glucuronidase and arylsulphatase. Separation of the water-soluble metabolites indicate that 50% are sulphate-conjugates, 15% are glucuronic acid conjugates and 35% glutathione or more polar conjugates. The binding levels of benzo[a]pyrene to DNA in cultured colon showed a unimodal distribution (56 cases). A slight difference ($p < .1$) in binding levels between non-tumorous tissues from cancer patients (40 cases) and tissues from non-cancer patients (16 cases) was observed, the binding level being highest in the latter. The binding level of benzo[a]pyrene to DNA also showed approximately 5-fold variation among the different anatomical segments of the colon from the same patient; the binding level was generally highest in the ascending colon. Co-incubation of the explants with benzo[a]pyrene and either taurodeoxycholic acid or lithocholic acid increased the binding levels of benzo[a]pyrene to DNA. A significant increase in the amount of water soluble metabolites was found when the explants were incubated with taurodeoxycholic acid; an increased level of trans-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene in the media was also observed when explants were co-incubated with either taurodeoxycholic acid or lithocholic acid.

AFB. was metabolized by cultured human colon. The binding level of AFB_1 to DNA was lower than observed with BP in the same patients. The major AFB_1-DNA-adduct formed was identified as 2,3-dihydro-2-(N^7-guanyl)-3-hydroxyaflatoxin B_1.

Cultured human colon mucosa was found to metabolize both acyclic and cyclic N-nitrosamines as measured by $^{14}C-CO_2$ formation and reaction of the activated moieties with cellular macromolecules. Dimethylnitrosamine and N-nitrosopyrrolidine were metabolized by explants from all patients studies. A positive correlation between binding of dimethylnitrosamine to DNA and CO_2-formation was observed. DMN alkylated DNA in both O-6 and N-7 position of guanine. However, most of the radioactivity was associated with an acid labile compound. High binding levels of N,N'-dinitrosopiperazine to protein without concommitant binding to DNA were detected. Inter-individual variation in both binding level to DNA and ability to metabolize the different N-nitrosamines was observed. The metabolies from incubation of human colon with NPy was separated using HPLC. There was evidence for both α- and β-oxidation.

Significance to Biomedical Research and the Program of the Institute: Chemical carcinogens which are metabolically activated and form carcinogen-DNA adducts by human colon may induce cancer in this tissue in humans. Determination of binding levels of carcinogens in a greater group could identify people at high risk for chemical induction of colonic cancer.

Proposed Course of the Project: Study in more detail of the metabolism of DMH and N-nitrosamines in particular N-nitrosopyrrolidine. The effect of various co- and anti-carcinogens on the metabolism of DMH will be investitigated in human colon. The relationship between the genetic control of DMH-carcinogenesis and the enzymatic activation will be investigated in animal models.

Publications

Autrup, H. N., Harris, C. C., Stoner, G. D., Jesudason, M. L., and Trump, B. F.: Binding of chemical carcinogens to macromolecules in cultured human colon. J. Natl. Cancer Inst. 59: 351-354, 1977.

Autrup, H., Harris, C. C., Fugaro, S., and Selkirk, J. K.: Effect of various chemicals on the metabolism of benzo[a]pyrene by cultured rat colon. Chem. Biol. Interact. 18: 337-347, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04489-02 EXP

PERIOD COVERED October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Explant Culture of Human and Rat Colon

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. Autrup	Staff Fellow	EXP, NCI
	C. Harris	Head, HTSS	EXP, NCI
	G. Stoner	Expert	EXP, NCI

COOPERATING UNITS (if any)

University of Maryland, School of Medicine, Baltimore, MD

LAB/BRANCH

Experimental Pathology Branch

SECTION

Human Tissue Studies Section (HTSS)

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.25	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Conditions for culturing explants of both human and rat colon have been developed. Rat colon has been maintained up to 63 days under these conditions, while human colon could generally be maintained up to 7 days; tissue from some patients have been maintained for 24 days. The viability of the tissue was determined by incorporation of radioactive precursors into DNA and protein, and by electron microscopy.

PHS-6040
(Rev. 10-76)

Objectives: To develop an explant culture system with human colon for carcinogenesis studies in this organ under controlled conditions.

Methods Employed: Explant cultures; electron microscopy; high resolution light microscopy; autoradiography; xenotransplantation.

Major Findings: Human colonic epithelium has been cultured as explants in a chemically defined medium for periods of 1 to 24 days. The viability of the explants was shown by the preservation of the ultrastructural features of the colonic epithelial cells and by active incorporation of radioactive precursors into cellular DNA and protein. A progressive decrease in the number of goblet cells, decrease in the depth of the crypts, and a change from a columnar to a cuboidal epithelium were observed. After 24 days in culture the colonic mucosa consisted of a single layer of cuboidal epithelial cells and a few glands. The ability to maintain colonic mucosa in culture was subject to both intra- and inter-individual variation. The explants were generally maintained up to 7 days under these conditions. Adult rat colon was maintained up to 62 days under similar conditions.

Significance to Biomedical Research and the Program of the Institute: A colon explant system has been developed which can be used for carcinogenesis studies and for studies of the pathogenesis of other colonic diseases.

Proposed Course of the Project: Improving the culture condition to be able to maintain the explant for a longer time. Attempts will be made to culture dispersed epithelial cells from rat and human colon. The effect of bile acids on cellular ultrastructure will be investigated.

Publications

H. Autrup, L. A. Barrett, F. E. Jackson, M. L. Jesudason, G. Stoner, P. Phelps, B. F. Trump, and C. C. Harris: Explant culture of human colon. Gastroenterology, 1978, In press.

H. Autrup, G. D. Stoner, F. Jackson, C. C. Harris, A. K. M. Shamsuddin, L. A. Barrett, and B. F. Trump: Explant culture of rat colon: A model system for studying metabolism of chemical carcinogens. In Vitro, 1978, In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04490-02 EXP

PERIOD COVERED October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Evaluation and Prevention of Carcinogenic Effects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Umberto Saffiotti, M.D. Chief, Experimental Pathology Branch EXP, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH Experimental Pathology Branch

SECTION Office of the Chief

INSTITUTE AND LOCATION NCI, NIH, Bethesda, MD 20014

TOTAL MANYEARS: 0.8	PROFESSIONAL: 1.6	OTHER: 0.2

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The primary objectives of this work are to examine current knowledge in the field of carcinogenesis and related fields and to identify criteria for the evaluation of carcinogenic effects of chemical and physical agents and for the prevention of cancer hazards in the human population.

Laboratory methods and biological models for the detection of carcinogenic activity of chemicals are examined and evaluated, with particular emphasis on in vitro models for carcinogenesis studies and animal models of carcinogenesis.

Criteria for the evaluation of carcinogen risk from environmental and occupation human exposures are developed.

PHS-6040
(Rev. 10-76)

Objectives: To examine current knowledge in the field of carcinogenesis and related fields and to identify criteria for the evaluation of carcinogenic hazards in the human population.

Methods Employed: Examination and evaluation of biological models, methods and findings used for the detection of carcinogenic activity. Analysis of results of carcinogenesis studies, including pathology, experimental design and statistical evaluations. Analysis of occupational and environmental exposure data. Analysis of regulatory, legislative and societal approaches. Development of critical reviews and documentation in environmental carcinogenesis. Organization of conferences and workshops to provide evaluation of current scientific bases of carcinogenesis studies. Preparation and editing of documentation of these topics. Participation in advisory groups for national and international ortganizations and for agencies of Federal Government.

Major Findings: Particular contributions were provided in the following areas:

1. Documentation on in vitro carcinogenesis methods: (a) Organization of a "Meeting on methods for carcingenesis tests at the cellular level and Their evaluation for the assessment of occupational cancer hazards", held at the Carlo Erba Foundation, Milan, Italy, December 1977. (b) Editing and publication of a book entitled "In Vitro Carcinogenesis. Guide to the literature, recent advances and laboratory procedures" (U. Saffiotti and H. Autrup, Eds.; NCI Carcinogenesis Techn. Rep. Series, 1978). (c) Review of organ culture methods in carcinogenesis studies (U. Saffiotti and C. C. Harris, in press).

2. Definition of criteria for carcinogen identification and risk assessment. In a series of papers presented at scientific conferences and now published the following general criteria were defined: (a) distinction of toxic effects into terminal toxic effects and self-replicating toxic effects; (b) classification and definition of carcinogenesis test results into the catergories of "positive", "negative under the conditions of observation" and "inconclusive"; (c) distinction of the process of risk evaluation from the process of benefits evaluation and from a third process of evaluation of technological alternatives for carcinogenic hazard prevention; (d) implications of etiological knowledge on ethics; (e) need for toxicology standards comparable to those required in forensic medicine; (f) the need for licensed laboratories for carcinogenesis testing; (g) the need for full public documentation of carcinogenesis data.

3. Review of state of knowledge and recommendation of criteria for the evaluation of the carcinogenic effects: (a) Air pollution (review published in 1978); (b) Pesticides (review in press).

4. Advisory activity to Agencies of the Federal Government on the evaluation of criteria for scientific policy in the regulation of environmental carcinogens. (a) Testimony and consultation at the request of the Assistant Secretary of Labor for OSHA on the proposed "Regulation of certain toxic materials. Identification, classification and regulation of toxic materials posing a potential occupational cancer risk to workers". (b) Evaluation and submission of testimony for administrative law hearing, at the request of the Office of the General Council, Food and Drug Administration, on the evaluation of the carcinogenicity of cylamate. (c) Participation, Subgroup (III B) (on Cancer Policy), Toxic Substances Strategy Committee, Council on Environmental Quality, Executive Office of the President. (d) Participation, Planning Committee for the President's Environmental Health Initiative, Executive Office of the President.

5. Invited participation to scientific and advisory meetings: Conference on Carcinogenic Risks and Strategies for Intervention, Internat. Agency for Research in Cancer, Lyon, France, Nov. 1977; Meeting on Methods for Carcinogenesis tests at the cellular level and their evaluation for the assessment of occupational cancer hazards, Carlo Erba Foundation, Milan, Italy, Dec. 1977; 31st Annual Symposium on Fundamental Cancer Research on Carcinogens: identification and mechanisms of action, Houston, TX, March 1978; Workshop on Methodology for Assessing Reproductive Hazards in the Workplace, Bethesda, MD, April 1978; The Medical Society of New Jersey, Governor's Conference, Atlantic City, NJ May 1978; New York Academy of Sciences International Conference on Public Control of Environmental Health Hazards, New York, NY, June 1978. Also participation in the scientific activities of the Society for Occupational and Environmental Health, as Vice-President.

Significance to Biomedical Research and the Program of the Institute: The national policies on environmental health and cancer prevention need to be based on sound scientific grounds. A solid and well documented basis of research data and evaluations is necessary in the develoent of criteria for a sound health protection policy.

The experience obtained by analyzing and coordinating different methodological approaches to the study of the carcinogenic process, at the human, animal, cellular and molecular level, provides a strong basis for identifying specific high priorities in public health and for further research.

Proposed Course of Project: Continuation of these activities.

Publications

Saffiotti, U. and Page, N. P.: Releasing carcinogenesis test results: Timing and extent of reporting. *Med. Pediatr. Oncol*., 3: 159-167, 1977.

Saffiotti, U.: Identifying and defining chemical carcinogens. In Hiatt, H., Watson, J. D., and Winsten, J. (eds.): Origins of Human Cancer, Cold Spring Harbor Conferences on Cell Proliferation. Cold Spring Harbor, New York, Cold Spring Harbor Laboratory, Vol 4: 1311-1326, 1977.

Saffiotti, U.: Scientific bases of environmental carcinogenesis and cancer prevention: developing an interdisciplinary science and facing its ethical implications. J. Toxicol. Environ. Health, 2: 1435-1447, 1977.

Saffiotti, U.: Carcinogenesis 1957-77: Notes for a historical review. J. Natl. Cancer Inst., 59: 617-622, 1977.

Saffiotti, U. and Autrup, H. (eds.): In vitro carcinogenesis. Guide to the literature, recent advances and laboratory procedures. NCI Carcinogenesis Techncial Report Series, No. 44. DHEW Publ. No. (NIH) 78-844, 1978. 316 pp.

Saffiotti, U.: In vitro carcinogenesis methods in relation to the development of carcinogenesis research. In Saffiotti, U. and Autrup (Eds.): In Vitro Carcinogenesis. Guide to the literature, recent advances and laboratory procedures. NCI No. (NIH) 78-844, 1978, pp. 1-20.

Harris, C. C., Saffiotti, U. and Trump, B. F.: Meeting Report. Carcinogenesis Studies in Human Cells and Tissues. Cancer Res., 38: 474-475, 1978.

Saffiotti, U.: Experimental approaches to the identification of environmental carcinogens. In Epstein, S. S. (Ed.): Environmental Determinants of Human Cancer. Springfield, Illinois, Charles C. Thomas Publishers (in press).

Saffiotti, U.: Experimental identification of chemical carcinogens, risk evaluation and animal-to-human correlations. Environ. Health Perspectives, 1978 (in press).

Saffiotti, U.: Review of pesticides carcinogenesis data and regulatory appraches. IARC-INSERM Symposium on "Carcinogenic Risks-Strategies for Intervention" IARC, Lyon, 1978 (in press).

Saffiotti, U. and Harris, C. C.: Carcinogenesis studies on organ cultures of animal and human respiratory tissues. In Carcinogens: Identification and Mechanisms of Action. 31st Annual Symposium on Fundamental Cancer Research, University of Texas, 1978 (in press).

Saffiotti, U.: Eziologia ed etica: Problemi di una nuova tossicologia ambientale. Med. Lavoro (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04491-02 EXP

PERIOD COVERED October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Quantitative studies on concurrent exposures to numerous carcinogens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Umberto Saffiotti	Chief, Experimental Pathology Branch	EXP, NCI
Other:	Jerry M. Rice	Head, Perinatal Carcinogenesis Section	EXP, NCI
	Winston D. Edwards	Visiting Scientist	EXP, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH Experimental Pathology Branch

SECTION Office of the Chief

INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	0.7	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

An experimental design is being developed for the concurrent exposure of appropriate biological targets to numerous carcinogens of different classes, each administered at doses which would be expected to produce marginal or undetectable effects in the chosen system. Quantitative exposure/response studies are addressed to test the hypothesis that many different carcinogens can act synergistically to induce a significant level of carcinogenic response when given concurrently at individually ineffective doses. The methods selected for these studies include in vitro assays for mutagenesis and neoplastic cell transformation and whole animal models. Analysis of dose-response relationships in carcinogenesis is conducted on data collected in a comprehensive literature survey.

PHS-6040
(Rev. 10-76)

Objectives: To determine whether a marked carcinogenic effect can result from the concurrent exposure to numerous different carcinogens, each administered at doses which would be expected to produce marginal or undetectable effects in the chosen target system; and to analyze dose-response relationships in carcinogenesis.

Methods Employed: Biological model systems include Salmonella mutation tests (Ames) and neoplastic transformation of Balb/c 3T3 mouse cells in culture (Kakunaga). Whole animal carcinogenesis studies are planned. Quantitative exposure protocols are developed. It is planned to extend the exposures to as many as 100 different carcinogens given concurrently. Quantitative analysis of the results will be performed.

Major Findings: The Salmonella mutation system (Ames) was set up and calibrated. Dose-response data were obtained, extending to the lowest detectable levels, using direct acting and metabolically activated carcinogens. The Balb/c 3T3 mouse fibroblast cell line system (Kakunaga) was used in preliminary studies for neoplastic transformation. Data on dose-response relationships in carcinogenesis were collected from a comprehensive survey. Their analysis was begun based on class of carcinogens, route, dose and number of administrations. Particular emphasis was given to studies of multiple exposures to different agents.

Significance to Biomedical Research and the Program of the Institute: Multiple concurrent exposures to many different carcinogens represent the actual conditions under which the human population is exposed to carcinogens. From prenatal life through childhood and adult life people are exposed to a large number, probably in the hundreds, of environmental carcinogens from different routes. Such "realistic" conditions of exposure have never been reproduced experimentally so far. The definition of the effect of this background low-level exposure to individual carcinogens, acting together with the exposure to many others, is expected to provide information on the mechanisms of carcinogenesis in tissues exposed to multiple hits by different chemicals and on the role of multiple synergisms in carcinogenesis. The role of high-level exposures to single carcinogens when superimposed to different kinds of background multiple exposures will also be clarified. Wide-ranging implications can be projected for the understanding of basic chemical biological interactions in carcinogenesis, for mathematical models for dose/response extrapolation and for the evaluation of carcinogenic hazards in public health policies.

Proposed Course of Project: Continuation.

Publications

An outline and discussion of this project was included in the following publication:

Saffiotti, U.: Identifying and defining chemical carcinogens. In Hiatt, H., Watson, J. D., and Winsten, J. (Eds.): Origins of Human Cancer. Cold Spring Harbor Conferences on Cell Proliferation. Cold Spring Harbor Laboratory, N.Y., Vol. 4: 1311-1326, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04504-06 EXP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Model Systems for the Study of Chemical Carcinogenesis at the Cellular Level

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. H. Yuspa	Head, In Vitro Pathogenesis Section	EXP, NCI
Other:	H. Hennings	Senior Scientist	EXP, NCI
	U. Lichti	Expert	EXP, NCI
	N. Colburn	Expert	EXP, NCI
	T. Bowden	Staff Associate	EXP, NCI
	M. Poirier	Chemist	EXP, NCI

COOPERATING UNITS (if any)

Dermatology Branch, DCB, NCI
Differentiation Control Section, Experimental Pathology Branch
Laboratory of Pulmonary Function and Toxicology, NIEHS

LAB/BRANCH

Experimental Pathology Branch

SECTION

In Vitro Pathogenesis Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 10	PROFESSIONAL: 6	OTHER: 4

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Mouse epidermal cell cultures and epidermal cell lines are utilized as models for studying mechanisms of epithelial carcinogenesis in vitro. Epidermal cells differentiate to produce normal keratin and retain epidermal-specific cell surface antigens in vitro yielding excellent markers for differentiation. Tumor promoters alter epidermal cell differentiation and induce certain cell strains to gain the ability to grow in agar. Tumor promoters also induce new synthesis of ornithine decarboxylase as well as prolong the half-life of the activity of this enzyme. Active metabolites of benzo[a]pyrene induce gaps in replicating strands of DNA; the ratio of gaps to bound BP molecules is one for the most carcinogenic metabolite and two for a less carcinogenic stereoisomer suggesting a difference in repair processes for each metabolite. Radioimmunoassay detection of carcinogen-DNA adducts is both highly specific and sensitive and can follow binding and removal of a specific DNA product as well as molecular alteration of the bound carcinogen.

PHS-6040
(Rev. 10-76)

Objectives: To study cellular and molecular changes during stages of chemical carcinogenesis through the use of unique in vitro model systems designed to simulate well studied in vivo models. Studies are directed to give insight into general changes occurring in mammalian cells during malignant transformation and specific molecular events which may be causative to the transformation process. Specific markers of the transformed phenotype are also being sought and mechanisms to prevent or reverse transformation are being studied.

Methods Employed: This laboratory has developed and utilized mouse epidermal cell culture as a major model to approach the stated objectives. Previous studies have shown that this model functions biologically in a highly analogous fashion to mouse skin in vivo. Human epidermal cells obtained from neonatal foreskins have also been adapted to growth in vitro in recent years. In vivo studies utilizing the two stage mouse skin carcinogenesis model and grafts of human skin onto nude mice are also employed.

A number of laboratory techniques are required to pursue the objectives. Morphology is followed by light and electron microscopy and histochemical staining. Macromolecular synthesis and growth kinetics are studies by biochemical and autoradiographic procedures. Cellular functions, including the production of specific differentiation products are monitored by enzyme assays and polyacrylamide gel electrophoresis. The progression to the malignant phenotype is monitored by growth rates, soft agar assay, production of plasminogen activator and injection of cells into nude or newborn mice. A number of immunologic techniques including cell surface antibody production, fluorescent staining, immunoprecipitation and mixed hemabsorption assays are being performed to recognize the normal or altered phenotype. Radioimmunoassay is used in studies of carcinogen-DNA binding and repair. In addition the chemical synthesis of DNA-carcinogen adducts is currently performed. Assays for post-replication repair and DNA molecular weight determinations utilize density gradient centrifugation.

Major Findings: The pursuit of this project has led to major new findings in three pertinent areas: 1. enhancement of model systems including improved culture methodology, transformation assay and identification of markers for normal and transformed cells; 2. new understanding of the mechanisms of initiation of carcinogenesis; 3. new information concerning the mechanisms of tumor promotion or progression. In addition continuing confirmation of in vitro results by in vivo experimentation has been obtained.

During the past year differentiating epidermal cell lines have been developed utilizing enriched culture medium and selective trypsinization procedures. These are epithelial in gross appearance and show typical characteristics of epidermis by electron microscopy. Keratinizing cells are shed into the medium and epidermoid cysts are produced upon injection of one such line into newborn syngeneic mice. This line does not produce tumors or grow in agar. A second line has similar characteristics and produces immunologically identifiable keratin. Adult mouse cells from carcinogen initiated animals have also been adapted to grow in culture but as yet lines have not developed. Human epidermal cells, isolated from neonatal foreskins, grow well on a collagen base

and can be passaged at least 4 times. Optimum growth conditions are now being defined. A number of additional cell lines with varying degrees of epithelial differentiation have been established by treatment with carcinogens or tumor promoters. Most are from a mouse strain which is susceptible to skin tumor formation. These are currently being utilizied to study tumor progression in vitro. In addition a collaborative study to establish an in vitro transformation model utilizing hamster epidermal cells is underway at the National Institutes of Environmental Health Sciences. Specific differentiation markers for epidermal cells have been identified in vivo and in cells in culture. Two major prekeratin proteins of M.W. 68,000 (K_1) and 60,000 (K_2) have been isolated and characterized. While K_2 appears to be present in cells of all stages of differentiation, the production of K_1 is associated with irreversible terminal differentiation and production of keratin filaments. Phorbol ester tumor promoters interfere with this process of differentiation by stimulating a subpopulation toward accelerated keratinization and inhibiting keratinization in a proliferating population. Antibodies produced against these differentiation proteins immunoprecipitate extracts of epidermal cells but not fibroblasts. Antibodies have also been produced utilizing intact mouse epidermal cells as antigen. These antisera, after appropriate absorption, recognize primary epidermal cells from mouse, hamster and human as well as mouse epidermal cell lines but not fibroblasts of any species utilizing the mixed hemabsorption assay. While end-titers are generally lower in transformed lines versus non-malignant lines, an absolute correlation of low reactivity with transformation could not be shown. On the other hand, growth in agar has been an excellent in vitro correlate to tumorigenicity in epidermal cell lines. Plasminogen activator secretion is a fair indicator of tumorigenicity with levels being highest in transformed lines; moderate levels are also noted in non-transformed lines however.

Studies to understand the mechanism of tumor initiation have focused on the interaction of carcinogens with DNA. A highly sensitive and specific radioimmunoassay was developed in this laboratory against the major guanosine adduct (dG-8-AAF) of the carcinogen acetylaminofluorene (AAF). This assay has now been utilized to study the fate of this adduct after carcinogen exposure to epidermal cells. After 1 hour of exposure, the total C_8 adducts represent 50-75% of total bound carcinogen as measured by binding of ^{14}C-AAF. By 24 hours about 40% of the bound C_8 adducts are removed, presumably by repair processes. Most if not all of the bound AAF is present in the deacetylated form (also recognized by the antiserum). Antibodies to other carcinogen-DNA adducts are currently being produced. The study of another aspect of carcinogen-DNA interaction, DNA repair has yielded important new information. Exposure of epidermal cells to the ultimate carcinogenic metabolite of benzo[a]pyrene, BP-diolepoxide 1, results in gaps in newly replicating DNA, presumably opposite bound sites on the parent strand since there is a 1:1 correlation between the number of bound BP molecules and the number of gaps. These gaps are ultimately closed by a post-replication repair process which is caffeine sensitive. Exposure of cells to the equally reactive but much less carcinogenic metabolite BP-diolepoxide II also produces gaps on the daughter strand but in a ratio of 2 gaps per bound BP molecule. This suggests that DNA

damage results in different effects for each of these metabolites both in replicative polymerase activity and possibly in repair potential. This difference may be related to the marked difference in carcinogenic potency of these compounds.

New information has also been obtained concerning the mechanism of tumor promotion, a potentially reversible process, and tumor progression, defining stages of increasing expression of the transformed phenotype. The regulation of ornithine decarboxylase (ODC) activity which is elevated shortly after exposure of epidermis to tumor promoters both in vivo and in vitro was studied utilizing inhibitors of macromolecular synthesis. Utilizing RNA and protein synthesis inhibitors it appears that both transcription and translation are essential for ODC elevation suggesting new enzyme synthesis occurs in response to promoter stimulation. However actinomycin D, at doses which inhibit only ribosomal RNA synthesis, superinduces ODC activity when given along with tumor promoters suggesting a repressor of enzyme activity which is highly sensitive to act. D may be present in mammalian cells. The half life of ODC enzyme activity in epidermal cells is prolonged 2-fold after treatment with phorbol ester tumor promoters. Actinomycin D or the steroid fluocinolone acetonide, which also superinduces promoter stimulated ODC, do not further increase enzyme half life. Thus a second level of control, the regulation of enzyme degradation, is also influenced by tumor promoters. The stimulation of cell proliferation by tumor promoters is affected in a number of ways by metabolic inhibitors which do not necessarily parallel the effects on ODC. Thus these two effects can be dissociated as has been previously described in this laboratory. Recent in vitro studies have also revealed that retinoids, potent inhibitors of tumor promotion in vivo, inhibit promoter stimulated ODC activity and proliferation. Similar findings have been reported in vivo. In an attempt to identify stages in progression to the tumorigenic phenotype, a number of epidermal cell lines have been developed. Many of these are non-tumorigenic and do not grow in agar. Of these, 3 lines have been identified which become agar positive after exposure to phorbol esters for 2 weeks. There is an excellent correlation between promoter potency in vivo and the ability to induce agar colonies in vitro. Responding lines are not necessarily stimulated to proliferate by phorbol esters and the transition can be inhibited by simultaneous exposure to retinoids but not steroids. Clones of cells converted to agar positivity by phorbol esters are permanently altered. These lines appear to represent a late stage in tumor progression and offer an opportunity to study a discrete step in the transformation process.

Studies have continued in vivo where confirmation or extention of in vitro results are required. A new model system, where human foreskins grafted to nude mice are exposed to carcinogens and/or promoters, offers a unique opportunity to extend experimental observations to human tissues. Preliminary results have demonstrated that phorbol esters produce hyperplasia in human skin grafts and papilloma formation on human skin can result. Furthermore the graft procedure makes nude mice more susceptible to skin tumorigenesis suggesting a role for immunostimulation in 2 stage carcinogenesis. Studies on

the mechanism whereby stimulated proliferation enhances initiation of carcinogenesis suggest that proliferation after carcinogen damage is pertinent to tumor enhancement. Such results implicate a fixation of a mutagenic event or involvement of a post-replication repair process in initiation.

Significance to Biomedical Research and the Program of the Institute: The great majority of human cancers are induced by chemicals and most of these tumors develop in epithelial cells. Animal models have been extremely useful for bioassay and mechanistic studies but suffer from complicated interactions between host and environment as well as variations in physiological balance which impede investigations at the cellular level. The use of cell culture systems, particularly those of epithelial origin, offers the opportunity to extend the present state of knowledge to a more basic and cellular level. In addition cell cultures ultimately should be useful as bioassay screening procedures to detect carcinogens and co-carcinogens in the environment. Most of our present basic concepts concerning the pathogenesis of cancer were developed from studies utilizing carcinogen painting on mouse skin. The irreversibility of initiation, the phenomenon of co-carcinogenesis and tumor promotion, the role of hyperplasia and metabolism are examples of such concepts. The development of a cell culture system for epidermal carcinogenesis has been a major advance toward extending our knowledge of mechanisms of carcinogenesis. Earlier efforts were directed to proving that epidermal cells in culture responded to carcinogens and promoting agents as in vivo. In almost every parameter studied this was the case. Differentiation, metabolism, proliferation, activation and binding of carcinogens, and promoter responses were highly analogous to in vivo. These findings enhanced the validity of any subsequent observations made in vitro. For the last several years the model system has been utilized to a much greater degree to ask questions about mechanisms of transformation, carcinogen and promoter interactions and the role of anti-carcinogenic agents such as retinoids and steroids. Long term growth of nontumorigenic and tumorigenic cell strains offers the opportunity to study the cancer phenotype and tumor progression. The role of differentiation in carcinogenesis can also be studied. Antigenic markers have become useful tools to detect normal and abnormal states and provide a rapid assay for differentiation. The use of cells from sensitive and resistant animals as well as carcinogen treated adult mice offers the opportunity to determine factors responsible for susceptibility. Greater understanding of the mechanisms of tumor promotion and the stages of tumor progression provides an opportunity to devise schemes for intervention in the process prior to the development of overt malignancy. Evidence for such a possiblity is already apparent from studies of steroid hormones and retinoids. DNA repair studies in a relevant system have enhanced our understanding of the role of damage and repair of the target cell genome with the possibility of intervening in the process. The development of an immunoassay for carcinogen-DNA adducts should have a number of important uses such as detection of carcinogens in human populations, following the repair of carcinogen damaged DNA and localizing the damaged site by a combination of immunological and morphologic techniques. Finally while bridging the gap between relevant animal models and in vitro

systems, this laboratory is simultaneously developing the analogous human tissue model in vitro. Thus ultimately a chain of systems will be available to determine the relevance of findings in any one model for the entire spectrum of models including the human.

Proposed Course of Project: Continued efforts will be directed toward development of improved models including the use of differentiating cell strains for in vitro transformation assays. Attempts to shorten the transformation process by selecting early transformants will be continued. A radioimmunoassay for keratin proteins K_1 and K_2 is being developed which should lead to quantitation of differentiation products under varying treatment conditions. An attempt to isolate the RNA message and ultimately the gene for these differentiation markers will be made so that the effect of initiators and promoters on specific gene expression can be studied. New antibodies are being developed against the major DNA adducts of aflatoxin and benzo[a]pyrene, two carcinogens with significant human exposure. If a radioimmunoassay can be devised utilizing these antibodies then studies to determine if these adducts are present in human material will begin. Studies will be continued on the mechanism of action of tumor promoters and stages of tumor progression. Particular emphasis will be placed on understanding the role of inhibitors of this process such as glucocorticoids and retinoids.

Publications

Yuspa, S. H.: Mouse epidermal cell cultures as an in vitro model for the study of chemical carcinogenesis. In Saffiotti, U. and Autrup, H. (Eds.): In Vitro Carcinogenesis, Guide to the Literature, Recent Advances and Laboratory Procedures. U.S. Government Printing Office, Washington, D.C., 1978, pp. 47-56.

Colburn, N.: Chemical transformation of epidermal cell cultures. In Saffiotti, U. and Autrup, H., (Eds.): In Vitro Carcinogenesis, Guide to the Literature, Recent Advances and Laboratory Procedures. U.S. Government Printing Office, Washington, D.C., 1978, pp. 57-64.

Poirier, M. C. and De Cicco, B. T.: The kinetics of excision repair synthesis induced by N-acetoxy-2-acetylaminofluorene and ultraviolet irradiation in human diploid fibroblasts. J. Natl. Cancer Inst. 59: 339-343, 1977.

Bowden, G. T., Hohneck, G., and Fusenig, N. E.: DNA excision repair in ultraviolet-irradiated normal and malignantly transformed mouse epidermal cell cultures. Cancer Res. 37: 1611-1617, 1977.

Colburn, N. H., Vorder Bruegge, W. F., Bates, J. R., Gray, R. H., Rosseen, J. D., Kelsey, W. H., and Shimada, T.: In vitro transformation of mouse epidermal cells: Correlation of growth in semi-solid medium with tumorigenicity. Cancer Res. 38: 624-634, 1978.

Hennings, H., Michael, D., and Patterson, E.: Croton oil enhancement of skin tumor initiation by N-methyl-N'-nitro-N-nitrosoguanidine: Possible role of. DNA replication. Proc. Soc. Exp. Biol. Med. 158: 1-4, 1978.

Schwartz, J. A., Viaje, A., Slaga, T. J., Yuspa, S. H., Hennings, H., and Lichti, U.: Fluocinolone acetonide: a potent inhibitor of mouse skin tumor promotion and epidermal DNA synthesis. Chem. Biol. Interact. 17: 331-347, 1977.

Lichti, U., Slaga, T. J., Ben, T., Patterson, E., Hennings, H., and Yuspa, S. H.: Dissociation of tumor promoter-stimulated ornithine decarboxylase activity and DNA synthesis in mouse epidermis in vivo and in vitro by fluocinolone acetonide, a tumor-promotion inhibitor. Proc. Natl. Acad. Sci 74: 3908-3912, 1977.

Yuspa, S. H., Lichti, U., Hennings, H., Ben, T., Patterson, E., and Slaga,, T. J.: Tumor promoter stimulated proliferation in mouse epidermis in vivo and in vitro: Mediation by polyamines and inhibition by the anti-promoter steroid fluocinolone acetonide. In Slaga, T. J., Sivak, A., and Boutwell, R. K. Boutwell (Eds.): Mechanisms of Tumor Promotion and Cocarcinogenesis, Raven Press; 1978, In press.

Lichti, U., Yuspa, S. H., and Hennings, H.: Ornithine and S-adenosylmethionine decarboxylases in mouse epidermal cell cultures treated with tumor promoters. In Slaga, T. J., Sivak, A., and Boutwell, R. K. (Eds.): Mechanisms of Tumor Promotion and Cocarcinogenesis, Raven Press, 1978, In press.

Colburn, N. H., Vorder Bruegge, U. F., Bates, J., and Yuspa, S. H.: Epidermal cell transformation in vitro. In Slaga, T. J., Sivak, A., and Boutwell, R. K. (Eds): Mechanisms of Tumor Promotion and Cocarcinogenesis, Raven Press, 1978, In press.

Hennings, H., Yuspa, S. H., Michael, D., and Lichti, U.: Modification of epidermal cell response to 12-O-tetradecanoyl-phorbol-13-acetate by serum level, culture temperature and pH. In Slaga, T. J., Sivak, A., and Boutwell, R. K. (Eds): Mechanisms of Tumor Promotion and Cocarcinogenesis, Raven Press, 1978, In press.

Slaga, T. J., Lichti, U., Hennings, H., Elgjo, K., and Yuspa, S. H.: Effects of tumor promoters and steroidal anti-inflammatory agents on newborn mouse skin in vivo and in vitro. J. Natl. Cancer Inst. 60: 425-431, 1978.

Poirier, M. C., Yuspa, S. H., Weinstein, I. B., and Blobstein, S.: Detection of carcinogen-DNA adducts by radioimmunoassay. Nature 270: 186-188, 1977.

Steinert, P. and Yuspa, S. H.: Biochemical evidence for keratinization of mouse epidermal cells in culture. Science, 1978, In press.

Bowden, G. T., Gresselbach, B., and Fusenig, N.E.: Postreplication repair of DNA in ultraviolet light irradiated normal and malignantly transformed mouse epidermal cell cultures. Cancer Res., 1978, In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04513-03 EXP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Metabolism of Chemical Carcinogens by Cultured Human Esophagus, Bronchus and Pancreatic Duct

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.25	0.5	

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Human bronchus, pancreatic duct and esophagus can be cultured for several weeks in a chemically-defined medium. This controlled experimental setting provides an excellent in vitro system to study the metabolism of chemical carcinogens including those found in tobacco smoke and the environment. Several classes of chemical carcinogens, polynuclear aromatic hydrocarbons, N-nitrosamines, hydrazines, and mycotoxins can be metabolically activated by human tissues.

The metabolic pathways leading to the formation of DNA adducts in cultured bronchus have been defined for benzo[a]pyrene, aflatoxin B_1, 1,2-dimethylhydrazine and dimethylnitrosamine. The adducts between these carcinogens and DNA in human bronchus are essentially the same as those found in experimental animals in which these chemicals are carcinogenic. When different people are compared a marked variation in binding levels are found, e.g., the inter-individual variation in the binding of BP to DNA in cultured human bronchus was 75-fold (100 patients). Nontumorous bronchi from patients with epidermoid carcinomas of the lung bind significantly more BP to DNA when compared to specimens from patients with either no cancer or adenocarcinomas of the lung.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04513-03-EXP

Objectives: To determine the metabolic pathways of chemical carcinogens in human target tissues. To measure the inter-individual variation in the metabolism of carcinogens.

Methods Employed: Explant culture of human tissues; quantitative high-resolution light microscopic autoradiography; isolation of cellular macromolecules; high-pressure liquid chromatography; enzyme assays.

Major Findings: Cultured human bronchial mucosa metabolically activate procarcinogens [polynuclear aromatic hydrocarbons: 7,12-dimethylbenz[a]anthracene (DMBA), 3-methylcholanthrene (MCA), benzo[a]pyrene (BP) and dibenz[a,h]anthracene (DBA); N-nitrosamines: dimethylnitrosamine (DMN), diethylnitrosamine (DEN), N-nitrosopiperidine (NPd), N-nitrosopyrrolidine (NPy) and N,N'-dinitrosopiperazine (DNP); a substituted hydrazine: 1,2-dimethylhydrazine (1,2-DMH); and a mycotoxin: aflatoxin B (AFB_1)] into forms that bind to cellular macromolecules including DNA.

The metabolism of two carcinogenic polynuclear aromatic hydrocarbons, BP and DMBA, was extensively studied in explants of human pancreatic duct and bronchus cultured in a chemically defined medium. In cultured human bronchial mucosa, activity of aryl hydrocarbon hydroxylase was inducible by both benz[a]anthracene and BP. Prior exposure of the bronchial explants to benz[a]anthracene altered the qualitative features of the metabolite profile of BP as analyzed by high-pressure liquid chromatography. The metabolite profiles of BP produced by normal-appearing bronchi from patients with lung cancer were also compared with those from patients without lung cancer. The profiles were similar except for an observed higher percentage of organic solvent-extractable metabolites formed by bronchi from the noncancer patients that eluted from the column as a single peak. This peak cochromatographed with both the 9,10-diol and a triol of BP. When compared to BP, binding levels of its weakly carcinogenic analog, benzo[e]pyrene, were more than 100-fold less. The major BP-DNA adduct formed in cultured human bronchial epithelium has been isolated by high pressure liquid chromatography and identified by elution order and circular dichroism spectra. The adduct results from the trans addition of the 2 amino group of guanine to the 10 position of (+)7R,8S,9R,10R enantiomer of BP-7,8-dihydrodiol-9,10-oxide. The removal of BP-DNA adducts was more rapid than BP-protein adducts. Our data suggest that the metabolic pathway of BP activation in human bronchus is similar to that found in experimental animals in which BP is carcinogenic.

An individual's risk of developing cancer may in part depend on the balance between activation and deactivation of procarcinogens. Initial studies have focused on the formation of BP-DNA adducts as a resultant of these competing metabolic processes. The binding of BP to DNA in cultured human bronchus was measured in specimens from 100 patients. A 75-fold variation in binding levels was observed which is similar in magnitude to that found in pharmacogenetic studies of drug metabolism and in our studies of BP metabolism in cultured human colon (Intramural Research Project Number Z01-CP-04488-02-EXP). We compared to patients without lung cancer and to those with adenocarcinoma of the

lung, nontumorous specimens from patients with epidermoid lung cancer had significantly higher levels of BP bound to DNA. Binding of BP to DNA was inhibited by 7,8-benzoflavone and by anti-oxidants (butylated hydroytoluene, disulfiram and vitamin E) but not altered by either nicotine or β-retinyl acetate. Binding of DMBA to DNA in pancreatic duct was consistently several-fold lower than binding levels in human bronchus.

Cultured human esophagus can enzymatically activate BP into metabolites that bind to macromolecules. The BP-DNA adduct(s) has not yet been identified. DMN and NPy are also activated to metabolites that bind to DNA.

Human bronchus was shown to metabolize and bind acyclic and cyclic N-nitrosamines found in the environment and in tobacco smoke. The metabolism of carcinogenic N-nitrosamines was studied in normal-appearing bronchial specimens. Explants of bronchi were cultured in a chemically defined medium for 7 days. N-Nitrosamines DMN, DEN, DNP, NPy, and NPd labeled with ^{14}C were each then added at 100 moles for 24 hours. Measurable CO_2 was formed by bronchial explants from: 1) DMN, DEN, and NPy in all 4 patients; 2) DNP in 3 of 4 patients; and 3) NPd in only 1 of 4 patients. In all bronchial specimens, metabolites of these N-nitrosamines and/or their metabolites bind to bronchial mucosal DNA and protein. Binding levels were higher to protein than to DNA. Binding levels of DNP were as high as those with the two acyclic N-nitrosamines DMN and DEN, but binding levels of NPy and NPd were lower. DMN methylated DNA at both O-6 and N-7 positions of guanine. Similar sites of methylation were found with 1,2-DMH.

As part of our survey of several different classes of chemical carcinogens, we found that AFB was activated by cultured human bronchus. The major adduct formed by the interaction of the electrophilic form of AFB_1 and DNA was 2,3-dihydro-2-(N^7-guanyl)-3 hydroxyaflatoxin B_1 with the guanyl-group and the hydroxy-group in trans position. The ratio of binding levels to DNA and to protein were higher than those found with BP. AFB_1 bound to DNA was removed at a faster rate than that bound to protein. Binding levels of AFB_1 to DNA were approximately one half of BP binding levels to DNA in cultured bronchus.

Significance to Biomedical Research and the Program of the Institute: Methodologies developed for and utilized in studies of carcinogenesis in experimental animals and cell cultures can be successfully extended to similar investigations in human cells and tissues. These investigations should eventually aid in identifying both chemical carcinogens and host factors determining susceptibility. Finally, these studies provide a much needed link between studies in experimental animals and man.

Proposed Course of the Project: Identify genetic and exogenous factors that alter the metabolism of environmental procarcinogens. Develop ultramicroassays of carcinogen metabolism so that metabolism can be studied in biopsy specimens. Correlate metabolism of chemical carcinogens between different target tissues within a single individual. Compare metabolism of chemical

carcinogens in target tissues and possible "detector" cells, i.e., monocytes and macrophages. To define water-soluble metabolites of BP and AFB_1 formed by cultured human tissues.

Publications

Harris, C., Autrup, H. and Stoner, G.: Metabolism of benzo[a]pyrene by cultured human tissues and cells. In Ts'o, P. O. P. and Gelboin, H. V. (Eds.): Polycyclic Hydrocarbons and Cancer: Chemistry, Molecular Biology and Environment, Academic Press, In press.

Harris, C., Autrup, H., Stoner, G., Yang, S., Leutz, J., Gelboin, H., Selkirk, J., Connor, R., Barrett, L., Jones, R., McDowell, E., and Trump, B.: Metabolism of benzo[a]pyrene and 7,12-dimethylbenz[a]anthracene by cultured human bronchus and pancreatic duct. Cancer Res. 37: 3349-3355, 1977.

Harris, C., Autrup, H., Stoner, G., McDowell, E., Trump, B., and Schafer, P.: Metabolism of acyclic and cyclic N-nitrosamines by cultured human bronchi. J. Natl. Cancer Inst. 59: 1401-1406, 1977.

Harris, C. C., Autrup, H. N., Stoner, G. D., Barrett, L. A., McDowell, E. M., Trump, B. F. and Schafer, P.: Binding of dimethylnitrosamine and 1,2-dimethylhydrazine to macromolecules in cultured human bronchi. Cancer Res. 37: 2309-2311, 1977.

Jeffrey, A. M., Weinstein, I. B., Jennette, K. W., Grzeskowiak, K., Nakanishi, K., Harvey, R. G., Autrup, H., and Harris, C.: Structures of benzo[a]pyrene-nucleic acid adducts formed in human and bovine bronchial explants. Nature 269: 348-350, 1977.

Harris, C., Autrup, H., van Haaften, C., Connor, R., Frank, A., Barrett, L., McDowell, E., and Trump, B.: Inhibition of benzo[a]pyrene binding to DNA in cultured human bronchi. In Nieburgs, H. (Ed.), Proc. 3rd Int. Symposium Cancer Detection and Prevention. Amsterdam, Excerpta Medica, 1978, 1359-1366 pp.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04567-05 EXP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Carcinogenesis Studies in Human Tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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SUMMARY OF WORK (200 words or less - underline keywords)
Model systems for studying carcinogenesis directly in human target tissues (bronchus, colon, breast, esophagus and pancreatic duct) have been developed to assess the: a) mechanisms of carcinogenesis in human cells; b) variation of carcinogenic susceptibility among individuals; and c) validity of the extrapolation of carcinogenesis data from experimental animals to the human situation. Metabolism of several classes of chemical procarcinogen is being studied in human tissues maintained under controlled experimental conditions. Investigations to date indicate that metabolic pathways and carcinogen-DNA adducts are qualitatively similar in human tissues and in tissues of experimental animals in which these chemicals are carcinogenic. A marked inter-individual variation in metabolism of benzo[a]pyrene has been found. A possible interaction between pulmonary macrophages and bronchial epithelium in the activation of benzo[a]pyrene during bronchogenic carcinogenesis has been identified. A human tissue- and cell-mediated mammalian mutagenesis assay has been developed. Chemical and physical carcinogens caused preneoplastic lesions in cultured and xenotransplanted human tissues.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04567-05-EXP

Objectives: To develop experimental systems to study carcinogenesis in human target tissues under controlled conditions. Such systems involve 3 major facets: 1) collection of viable human tissues; 2) in vitro maintenance of epithelial cells in these tissues in cell and organ cultures; and 3) xenotransplantation of human tissues into immunodeficient animals.

Methods Employed: Human tissues are obtained at either surgery or "immediate" autopsy. The tissue is maintained in culture and xenotransplanted into athymic nude mice. These tissues are exposed to chemical carcinogens in vitro and/or as a xenograft. The morphological lesions caused by carcinogens are examined by light and electron microscopy. Binding of carcinogens to macromolecules in epithelial cells is measured by biochemical and autoradiographic methods, which were previously developed, in part, in studies of animal models.

Major Findings: The methodology for each of the three major technical facets of this program (collection of tissues, explant culture and xenotransplantation) has been refined. The maintenance of human tissue (bronchus, colon, peripheral lung, breast, esophagus and pancreatic duct) in controlled experimental settings has allowed a series of interlocking studies including: 1) the metabolism of chemical carcinogens by human tissues and cells; 2) mutagenesis in mammalian cells (V-79 Chinese hamster cells) mediated by human tissues and cells; and 3) induction of epithelial lesions by chemical and physical carcinogens. Significant progress has been made in each of the above areas of investigation. Several classes of chemical carcinogens including many of those found in tobacco smoke as well as the environment are metabolically activated into forms that bind to cellular DNA. The major adducts between DNA and several chemical carcinogens (benzo[a]pyrene, aflatoxin B_1, dimethylnitrosamine and 1,2-dimethylhydrazine) have been isolated and identified. A marked inter-individual variation in levels of carcinogen-DNA adducts has been found. For example, the variation in binding levels of benzo[a]pyrene to DNA were 75-fold for bronchus (100 patients) and 100-fold for colon (57 patients). The relationship between formation of carcinogen-DNA adducts and individual's oncogenic susceptibility to the carcinogen is as yet not known. These biochemical studies of carcinogen binding to DNA are being correlated with biological studies, e.g., human tissue-mediated mutagenesis. Both benzo[a]pyrene and its proximate carcinogen, 7,8-diol, are enzymatically activated by human bronchus, macrophages and monocytes into metabolites that cause mutations in V-79 cells. While direct proof is as yet lacking it seems prudent to consider chemicals as being carcinogenic in humans if they 1) are carcinogenic in experimental animals; 2) form adducts with DNA in human epithelial cells; and 3) are converted by human tissues and cells to mutagens in experimental mutagenesis assays.

Morphological, cytochemical and immunological methods to identify in situ and cultured normal and neoplastic human epithelial cells have been refined. The pathogenesis of lung cancer in animal model has been shown to be morphologically similar to that found in humans.

Studies in progress suggest that carcinogens cause basal cell hyperplasia and atypical squamous metaplasia in cultured and in xenotransplanted human bronchial epithelium. The biological fate of these preneoplastic lesions is being determined.

The various facets of these studies are described in detail (Intramural Research Project Numbers Z01-CP-04485-01-EXP, Z01-CP-04486-01-EXP, Z01-CP-04487-02-EXP, Z01-CP-04488-02-EXP, Z01-CP-04489-02-EXP, Z01-CP-04513-03-EXP, and Z01-CP-04517-02-EXP. These investigations are conducted in collaboration with NIH contractors, University of Maryland (N01-CP-43237), Litton Bionetics (N01-CP-43274) and Washington Veterans Administration Hospital (Y01-CP-60204). Surgical and autopsy specimens are provided to NCI and to Litton-Bionetics by Washington Veterans Administration Hospital and by University of Maryland. Methods to culture explants of human tissues were developed in parallel at the University of Maryland and the NCI. Methods to culture human epithelial cells are being established at the NCI. Investigations concerning the metabolism of chemical carcinogens and the development of the human tissue mediated mutagenesis assay were conducted primarily at the NCI. Cultured tissues are exposed to carcinogens at the NCI and at the University of Maryland. Human tissues are implanted into athymic nude mice at Litton-Bionetics.

Significance to Biomedical Research and the Program of the Institute: The extrapolation of carcinogenesis data from studies utilizing experimental animals in man presents several complex problems. One approach to provide a link between these experimental data and human cancer is the development of an experimental system to study carcinogenesis in important human target tissues. Such a system could aid in the identification of individuals who are highly susceptible to environmental carcinogens, and methods of intervention could be tested in this experimental system prior to clinical trials in man.

Proposed Course of Project: Attempts are continually being made to improve each interlocking facet of this program. Studies have been initiated to develop cell cultures derived from human bronchial and pancreatic epithelial cells. These cultured cells will be characterized by both morphological,a immunological and biochemical methods. Attempts to transform the epithelial cells with chemical carcinogens will be made. Correlations will be made among the formation of carcinogen-DNA adducts in human tissues, mutations in V-79 cells mediated by human tissues and morphological lesions in cultured human epithelia caused by chemical carcinogens. Responses to chemical carcinogens in cultured human tissues will be compared to those found in human pathology and in animal models.

Publications

Harris, C.: Respiratory carcinogenesis. In Straus, M., Grune, and Stratton (Eds.): Lung Cancer: Clinical Diagnosis and Treatment. Grune and Stratton, Inc., New York, NY, 1977, pp. 1-17.

Harris, C., Autrup, H., Stoner, G., and Trump, B.: Model systems using human lung for carcinogenesis studies. In Harris, C. (Ed.): Pathogenesis and Therapy of Lung Cancer. New York, M. Dekker Inc., 1978, In press.

Harris, C., Autrup, H., Stoner, G., Valerio, M., Fineman, E., Schafer, P., Barrett, L., McDowell, E., Trump, B.: Explant culture and xenotransplantation of human bronchi. In Saffiotti, U. and Autrup, H. (Eds.): In Vitro Carcinogenesis, NCI Carcinogenesis Technical Report Series No. 44, DHEW Publication No. (NIH) 78-844, Washington, D.C., U. S. Government Printing Office, 1978, 144-153 pp.

Meadows, A. T., D'Angio, G. J., Mike, V., Banfi, A., Harris, C., Jenkin, R. D. T., and Schwartz, A.: Patterns of second malignant neoplasms in children. Cancer 40: 1903-1912, 1977.

Harris, C. (Ed.): Pathogenesis and Therapy of Lung Cancer. New York, M. Dekker, Inc., 1978, In press.

Harris, C. C., Saffiotti, U., and Trump, B. F.: Meeting report: Carcinogenesis studies in human cells and tissues. Cancer Res. 38: 474-475, 1978.

McDowell, E. M., Barrett, L. A., Glavin, F., Harris, C. C., and Trump, B. F.: Studies of human bronchial epithelium I. Normal. J. Natl. Cancer Inst., 1978, In press.

Trump, B. F., McDowell, E. M., Becci, P. J., Barrett, L. A., Glavin, F., Kaiser, H., and Harris, C. C.: Studies of human bronchial epithelium. II. Histogenesis of epidermoid metaplasia and carcinoma in situ. J. Natl. Cancer Inst., 1978, In press.

McDowell, E. M., McLaughlin, J. S., Merenyl, D. K., Kieffer, R. F., Harris, C. C., and Trump, B. F.: Studies of human bronchial Epithelium. III. Histogenesis of lung carcinomas. J. Natl. Cancer Inst., 1978, In press.

Saffiotti, U., and Harris, C. C.: Carcinogenesis studies on organ cultures of animal and human respiratory tissues. In M.D. Anderson Hospital and Tumor Institute Monograph, Carcinogens: Identification and Mechanisms of Action, 1978, In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04687-07 EXP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Transplacental and Neonatal Carcinogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. M. Rice	Senior Scientist	EXP, NCI
Other:	J. Berman	Staff Fellow	EXP, NCI
	B. Chen	Research Chemist	EXP, NCI
	W. Ching	Visiting Fellow	EXP, NCI
	P. Donovan	Chemist	EXP, NCI
	W. Edwards	Visiting Associate	EXP, NCI
	C. Wei	Visiting Fellow	EXP, NCI
	L. Keefer	Section Head	CMT, NCI
	P. Roller	Research Chemist	CMT, NCI
	W. T. London	Section Head	NINCDS, NIH

COOPERATING UNITS (if any)

NINCDS

LAB/BRANCH

Experimental Pathology Branch

SECTION

Perinatal Carcinogenesis Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 11.0	PROFESSIONAL: 6.0	OTHER: 5.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The roles of metabolism, immune processes, DNA repair, and other factors related to susceptibility to transplacental and organ-specific carcinogenesis in rodents and primates are investigated, with emphasis on nitrosamine and aromatic hydrocarbon carcinogens and utilizing both whole animal and in vitro culture techniques.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04687-07-EXP

Objectives: To develop experimental animal models for human cancers of childhood, and to study the greater sensitivity of fetal and neonatal animal tissues to chemical carcinogens, as well as the roles of factors other than the carcinogens themselves in enhancing or suppressing chemical carcinogenesis in experimental animals during intrauterine life and infancy and in specific organ systems in adults.

Methods Employed: Carcinogenic and radio-labeled organic compounds are synthesized as necessary. Animals of various species and strains, including nonhuman primates, are exposed to these compounds at different times during preand postnatal life, and the carcinogenic response in various organs is evaluated histologically. Short and long-term cell and organ culture techniques are used where necessary to explore the role of host defense mechanisms in resistance to carcinogenesis, and the role of differentiation processes in determining the histological features and biological behavior of transplacentally induced tumors. Surgical procedures, including skin grafting, adult thymectomy, etc., are used in evaluating antitumor defense mechanisms of the intact animal, and organic and biological chemical techniques are employed to determine the effects of modifications of chemical carcinogen structure and of metabolic factors on the response of host tissues to carcinogens. Standard biochemical procedures (alkaline sucrose gradient ultracentrifugation; in vitro enzyme-mediated protein synthesis from DNA templates; colorimetric assays of enzyme activity) and autoradiography are employed to investigate the fidelity and ontogeny of DNA repair capability in the developing fetus subsequent to carcinogen-induced damage. Computer techniques are used to estimate number-average and weight-average molecular weight of cellular DNA's from density gradients after exposure to alkylating agents. Histochemical demonstrations of enzymes and specific cell products are used to evaluate differentiation in, and to characterize cultures of, organs and cells from normal and neoplastic tissues and from fetal and adult animals.

Major Findings: Previous studies on carcinogenesis by ethylnitrosourea (ENU), and diethylnitrosamine (DEN) in the Old World monkey, Erythrocebus patas, have been continued and expanded. Repeated IV injections of ENU have induced malignant mesenchymal tumors of the blood vessels, bone, skeletal muscle, and lymphoreticular tissues, and carcinomas of the ovary, colon, and small intestine in juvenile and young adult monkeys of both sexes after latencies of 2-4 years from the beginning of treatment. Transplacental exposures to lower total doses of ENU have now yielded 11 tumors in 10 offspring; 8 of these became clinically evident during the first postnatal year, and the mean latency has been much shorter than in juvenile animals given 3-5 times the prenatal dose. Prenatal life is clearly a period of greatly increased susceptibility to carcinogens in this primate species, in accordance with the results of similar studies in rodents. However, the tumors induced transplacentally in primates resemble pediatric tumors of man, rather than those of adult life, and have become clinically evident during infancy, rather than adult life, as in rodents. The ability of the patas monkey to excise O-6 alkylation products from DNA of different organs after perinatal exposure to ENU-C^{14} or its methyl homolog, methylnitrosourea-H^3, has been studied in collaboration with German

investigators in Freiburg. O-6 methylguanine in guanine is rapidly removed from DNA in liver, but persists in brain, after treatment with MNU, and similar results have been obtained with ENU. This is exactly the same pattern seen in both rats and mice. It has been suggested that ability to remove these reaction products serves as a defense against carcinogenic damage, as the first step in effective DNA repair. The fact that both NMU and ENU effectively induce tumor of brain, but not liver, in rats contrasts with our finding of tumors of liver, but not brain, in monkeys, and with an enormous predominance of liver over brain tumors in mice given these agents. It is apparent that this excision process is not predictive per se of differences in susceptibility to carcinogenesis among various organs in different species.

Some female patas which received ENU beginning early in pregnancy at various dosage levels, including one which received only 2 injections of 0.1 mmole/kg, have subsequently either aborted their pregnancy or delivered a healthy full-term infant, and then a few months later have died suddenly of exsanguinating hemorrhage. The 7 animals in this category have all had multiple pulmonary metastases and diffuse endometrial infiltrates of choriocarcinoma, a neoplasm of trophoblastic tissue not systemically inducible in rodent species and for which no model currently exists. This result indicates that pregnancy is, for the gravid female as well as for her conceptus, a period of uniquely heightened susceptibility to carcinogens.

Further carcinogenesis studies in CD rats treated with methyl(acetoxymethyl)-nitrosamine (DMN-OAc) beginning at 5 weeks of age confirm that DMN-OAc behaves much like a very potent direct-acting agent. Subcutaneous administration produces a variety of tumors of dermal and mesenchymal origin. Intravenous administration produces tumors of lung, Zymbal's gland and nervous system including many endocardial neurinomas. Intragastric administration produces tumors of squamous and glandular gastric epithelium. Intraperitoneal administration, as shown previously, produces a high yield of intestinal neoplasms. In addition, numerous angiosarcomas, abdominal schwannomas, peritesticular mesotheliomas and mesothelial proliferative lesions were observed in rats administered DMN-OAc intraperitoneally. One case of a schwannoma found in the uterus of a rat treated with DMN-OAc intraperitoneally contained nests of proliferating granular cells identical cytologically, histochemically and ultrastructurally to the granular cells of "myoblastoma". This case served as evidence for the neuroectodermal origin of granular cell myoblastoma.

As groundwork for our study of the effects of embryonic development on the carcinogenic process, a number of techniques have been established for culturing fetal organ rudiments and for producing primary and/or established monolayer cultures of fetal epithelial organs. Rudimentary organs have been found to dissociate readily in the presence of a chelating agent (EDTA), and in the absence of proteolytic agents, in distinction to the situation with adult organs where proteolytic digestion is required for tissue dissociation and successful culturing. The rudimentary organs, as judged by electron

microscopy, are essentially devoid of desmosome functions. The role of desmosomes in expression of the epithelial phenotype in vivo and in cell culture is currently being explored.

As there are no characterized cell lines of adult rat kidney origin or rat nephroblastoma origin extant with which to compare our cultures of embryonic rat kidney, the American Type Culture Collection has supplied a morphologically epithelial line derived from pig kidney and a totally undescribed line derived from a human nephroblastoma (Wilms tumor). These have been characterized by several criteria. By electron microscopy, both lines were typically epithelial, with desmosome structures connecting polygon-shaped cells which often formed duct-like arrangements. The pig kidney line had a very pronounced basal lamina around individual cells and a rich network of microvillar projections suggesting properties of tubular epithelium. Enzyme histochemistry revealed that both lines demonstrated gamma glutamyl transpeptidase (GGTP) activity, which in the kidney is characteristic of differentiated proximal tubular epithelial cells. In addition, pig kidney and Wilms cells both had histochemically demonstrable 5'-nucleotidase and acid phosphatase activities. Wilms cells were positive for leucine aminopeptidase. All enzymes tested were shown to be present in rat kidney, primarily in the tubular epithelial cells. To our knowledge this represents the only characterization of either an adult kidney epithelial line or a nephroblastoma line. In addition, we have developed a technique for producing primary epithelial cell cultures from adult rat kidney. Cells in primary culture maintain an epithelial morphology (as judged by light microscopy and electron microscopy) for several weeks, after which most growth ceases and the primary culture is lost except for a few variant cells which continue to divide for at least six months. The variant surviving cells have a fibroblast-like morphology. The primary epithelial cells and the long-term cultured variant cells react positively for GGTP activity. The life of the primary culture can be dramatically extended by adding 10 mM ammonium acetate to the growth media.

As yet, no enzyme has been found exclusively or primarily in fetal kidney. A continuing effort to manipulate morphology and differentiation of normal and neoplastic renal tissue through media additives is under way. Mouse kidneys develop purely epithelial, well-differentiated tumors of adult type in response to transplacental carcinogens, while rat kidneys generate adult epithelial, nephroblastic, and undifferentiated (blastemal cell) tumors; the ability of rat and mouse renal tumor cells to respond to differentiation signals and the influence of the latter on their morphology, growth, and biologic behavior are being investigated. Work has continued on maintaining kidney rudiments in organ culture. To date, optimal maintenance of rudimentary rat kidney (15 gestation day) is achieved by growing kidneys on gelfoam sponge on a rocker assembly in 5% CO_2 and atmospheric oxygen in media supplemented with hydrocortisone, insulin and 10% fetal bovine serum. Organs survive with growth and differentiation and without necrosis for one week.

In order to demonstrate a functional property of cells in culture so that factors which alter the expression of differentiated phenotypes in homogeneous,

defined populations of cultured cells can be studied, a number of fibroblast cell lines and epithelial cell lines derived from tumors and from normal adult and embryonic tissues have been examined for the production of collagen in culture. When supplemented with ascorbic acid (50 μg/ml), all fibroblast lines produced collagen stainable by silver impregnation. Additionally, many of the epithelial lines produced collagen when supplemented with ascorbic acid. The most efficient producer of collagen was a line of demonstrated epithelial origin derived from rat liver. Analysis of the collagen types produced showed that Type III was predominant, with a minor amount of Type IV and a trace of Type I collagen. Type III collagen is found in the sinusoidal reticulin in normal liver and is the predominant collagen found in cirrhotic liver. The finding that liver epithelial cells in culture produce Type III collagen strongly indicates that the hepatocyte is the source of sinusoidal reticulin and cirrhotic collagen *in vivo*, and that the liver epithelial culture system may be an appropriate device for the study of collagen synthesis in liver.

Both MNU and ENU are inhibitory in an *in vitro* system in which β-galactosidase is synthesized from bacterial lac operon DNA. Under comparable conditions methylnitrosourea (MNU) is more inhibitory than ethylnitrosourea (ENU). When these agents were added at various times after the beginning of incubation, the inhibition was increasingly less effective, depending on the mole ratio of the carcinogen to DNA phosphate. For example, when (MNU: phosphate) was 18:1 the remaining β-galactosidase activities were 30, 31, 52 and 100% of the control for 0, 2, 5 and 10 min delayed addition respectively. These results suggest that these alkylnitrosoureas are most inhibitory early when mRNA is being synthesized. Using MNU or ENU treated DNA as template, the activity of the newly synthesized enzyme decreased with increasing concentration of the alkylnitrosourea. MNU reacted rapidly and extensively with DNA to inhibit its function, while ENU reacted much more slowly. Studies on the alkylation of DNA by H^3-MNU show a linear relationship between the amount of DNA alkylation and the concentration of MNU, and that the inhibition of the DNA gene function depends on the amount of the alkylation of DNA. Both the MNU and ENU treated DNA sedimented more slowly than the control DNA in alkaline sucrose gradients whenever enzyme synthesis was demonstrably inhibited. The synthesis of carbonyl labeled MNU and its use to investigate the correlation between DNA carbamoylation and the fidelity of gene function is under investigation.

A study of the damage inflicted by alkylating agents on cells of different organ systems at various stages of development has been undertaken to compare the efficiency of excision repair of DNA with organ-specific transplacental carcinogenesis in various species. Primary cultures of fetal brain, kidney, and liver were established, labeled with thymidine-H^3, and examined for DNA breakage and repair by ultracentrifugation after exposure in vitro to methyl methanesulfonate (MMS).

When 6 mM MMS was incubated with the rodent fetal cells in culture for 20 minutes, DNA single strand breaks were observed. DNA breakage was maximal 40 minutes later. Increased concentrations of MMS caused increased DNA

breakage and cytotoxicity. Most (approximately 80%) of the breaks repaired in fetal rat liver, mouse liver and brain 24 hours after MMS treatment. Only fetal rat brain showed significantly less repair (around 40%). This correlates with the high susceptibility of this organ to carcinogenesis by alkylating agents, but does not explain the susceptibility of mouse liver and resistance of rat liver to these compounds. The molecular weight range of single stranded DNA in these studies varied from 140 to 227 x 10^6 daltons. A fluorescent method for DNA determination is being adapted to measure DNA when less than 1 μg is present in alkaline sucrose solutions, allowing extension of these studies directly to fetal tissues without the necessity for cell culture or radioactive labeling procedures.

Significance to Biomedical Research and the Program of the Institute:
Research for animal models of human childhood neoplasms should provide an insight into the types of causative agents and modes of exposures responsible for childhood cancer. It is to be expected that natural selection would tend to eliminate genotypes in the human population which predispose individuals to the development of fetal neoplasms before attaining reproductive age, yet the incidence of tumors in childhood is relatively constant. Epidemiological studies have pointed to the occurrence of childhood neoplasms in association with certain types of congenital malformation which are non-inherited, and suggest that environmental agents, alone or in combination, may play a role in the induction of such neoplasms. The inducibility of tumors very similar to the pediatric tumors of man by chemical carcinogens in laboratory rodents and primates further supports this view. Most tumors induced transplacentally in rodents are of adult types and appear during adult life in individuals exposed in utero, resembling human experience with diethylstilbestrol. The ENU studies in patas monkeys will provide experimental data indicating whether adult or pediatric tumor types are more likely to develop in at least one species of primate in response to carcinogenic exposure in utero, and thus whether chemical carcinogens are likely to be involved in the prenatal genesis of pediatric or adult types of tumors in man. The demonstration of the inducibility of uterine choriocarcinoma by chemical carcinogens, at low exposure levels, further defines the nature of the prenatal hazard from chemical carcinogens, and further illustrates the importance of preventing human exposure to carcinogenic chemicals during pregnancy in either the workplace or environment.

Studies in rodents have shown that a fetus may be up to 4 orders of magnitude more susceptible to carcinogens than an adult of the same species, strain and sex, yet the precise reason for this enhanced vulnerability are not clearly understood. Moreover, in rodents, many tumors that develop as a consequence of exposure of incompletely differentiated cells to chemical carcinogens are morphologically identical to those inducible in adults, suggesting that the fundamental damage inflicted on such cells does not preclude subsequent programmed differentiation. The possibility that fetal cells may be deficient in activity or fidelity of DNA repair processes is a major definable physiologic function which might contribute to enhanced fetal susceptibility to these agents. The fact that differentiation overrides neoplastic transformation in

a given organ system (e.g., kidney) of certain species, but not others, provides a route to exploration of the basic nature of cellular differentiation in the control of neoplastic growth.

Carcinomas of the gastrointestinal tract and pancreas comprise the largest source of cancer morbidity in the adult population of the United States. Existing "intestine-selective" carcinogens are much less specific than is desirable. The high specificity of methyl(acetoxymethyl)nitrosamine for intestinal mucosa provides a new carcinogen well suited to studies of physiological and biochemical factors significant in development of intestinal carcinoma, including the roles of sex and genetic background as determinants of susceptibility.

Proposed Course of the Project: Direct and transplacental carcinogenesis by DMN-OAc and its ethyl homolog, DEN-OAc, will be explored further to define the effects of different doses and routes of administration on different strains of rats, mice, and hamsters. The metabolism and pharmacodynamics of distribution and excretion, especially the role of bile, will be studied using DMN-OAc-^{14}C.

Patas monkeys exposed to ENU or DEN either transplacentally or directly (after weaning) should continue to be observed for the development of tumors. In vivo studies should emphaszie further refinement of definition of periods of maximal prenatal susceptibility to direct-acting vs. enzyme-activated transplacental carcinogens. Pharmacodynamic studies will be continued to further study maternal-fetal distribution and tissue/organ localization of ENU-^{14}C in this species in comparison with rats and mice. Comparative DNA repair capacities of primate and rodent tissues after ENU treatment will be studied further, and extended to modalities of repair other than 0-6 alkylation product elimination.

Rat renal "blastemal cell" tumors will continue to be studied in organ culture to determine whether or not the morphologically mesenchymal tumors can be induced to form characteristic renal tubules and glomeruli or the enzymes characteristic of renal epithelium, thus confirming the relation of such neoplasms to nephroblastomas. Once the model is established, it should be used to determine the effects of manipulations of physiological parameters, inclding hormonal balance and immunological responsiveness, on the genesis and behavior of these tumors.

The possible role of DNA repair processes in modifying susceptibility of differentiating tissues to carcinogens should be actively investigated by both density gradient ultracentrifugation and autoradiographic techniques. The separate question of fidelity of repair of lesions in DNA inflicted by different classs of mutagenic and carcinogenic agents should first be explored in a simple, well-defined microbial system, with subsequent extension to mammalian systems if the gene product analysis approach appears promising. In particular, the β-galactosidase system will be used to determine whether no enzyme

or altered enzyme is synthesized from DNA damaged by alkylating agents, and whether the bacterial host cell is capable of accurately repairing this damage.

Publications

Martin, W. J., Gipson, T. G., Conliffe, M., Friedman, R. J., Dove, L., and Rice, J. M.: Common tumor-associated transplantation alloantigen detected on a proportion of lung tumors induced transplacentally in several strains of mice. Transplantation 24: 294-296, 1977.

Vesselinovitch, S. D., Koka, M., Rao, K. V. N., Mihailovich, N., Rice, J. M.: Prenatal multicarcinogenesis by ethylnitrosourea in mice. Cancer Res. 37: 1822-1828, 1977.

Sly, D. L., London, W. T., Palmer, A. E., and Rice, J. M.: Disseminated cryptococcosis in a patas monkey (Erythrocebus patas). Lab. Animal Sci. 27: 694-699, 1977.

San, R. H. C., Rice, J. M., and Williams, G. M.: Lack of correlation between plasminogen activating factor production and tumorigenicity in rat liver epithelial cells. Cancer Letters 3: 243-246, 1977.

Rice, J. M., Joshi, S. R., Shenefelt, R. E., and Wenk, M. L.: Transplacental carcinogenic activity of 7,12-dimethylbenz[a]anthracene. In Jones, P. W., and Freudenthal, R. I. (Eds.): Carcinogenesis, Vol. 3. Polynuclear Aromatic Hydrocarbons. 1978, Raven Press, New York, pp. 413-422.

Joshi, S. R., Rice, J. M., Wenk, M. L., Roller, P. P., and Keefer, L. K.: Selective induction of intestinal tumors in rats by dimethylnitrosamine, an ester of the presumed reactive metabolite of dimethylnitrosamine. J. Natl. Cancer Inst. 58: 1531-1535, 1977.

Ward, J. M., Rice, J. M., Roller, P. O., and Wenk, M. L.: Natural history of intestinal neoplasms induced in rats by a single injection of methyl (acetoxymethyl)nitrosamine. Cancer Res. 37: 3046-3052, 1977.

Rice, J. M.: Carcinogenesis: a late effect of irreversible toxic damage during development. Environmental Health Perspectives 18: 133-139, 1976.

Rice, J. M.: Comparative aspects of transplacental carcinogenesis. Comp. Path. Bull. 9 (4): 1 & 4, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04798-08 EXP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Role of Vitamin A in Control of Epithelial Differentiation and Carcinogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other:	C. S. Silverman-Jones	Microbiologist	EXP, NCI
	S. Adamo	Visiting Fellow	EXP, NCI
	W. Sasak	Visiting Fellow	EXP, NCI
	S. H. Yuspa	Senior Scientist	EXP, NCI
	N. H. Colburn	Expert	EXP, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Experimental Pathology Branch,

SECTION
Differentiation Control Section

INSTITUTE AND LOCATION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In an in vitro mouse epidermal cell carcinogenesis system, derivatives of retinol and retinoic acid were found to prevent the expression of neoplastic transformation induced by phorbol esters. The least active compound was found to be anhydroretinol, a retinoid with little vitamin A activity. Retinylphosphate and retinoic acid were the most active compounds. Cultured mouse epidermal cells were found to synthesize retinylphosphate and retinylphosphate-mannose. These products were characterized by their chromatographic behaviour and hydrolysis in mild alkali. This treatment released anhydroretinol from the phosphorylated vitamin. Treatment of cultured epidermal cells with excess retinol (4×10^{-5} M) greatly enhanced galactose and mannose incorporation into glycoconjugates without effect on total RNA synthesis or glucose utilization.

PHS-6040
(Rev. 10-76)

Objectives: To study the mode of action of vitamin A in the prevention of epithelial carcinogenesis and in maintenance of normal epithelial cell differentiation and growth in mammals.

Methods Employed: (a) Retinoids were prepared by published procedures or obtained from the Hoffmann-LaRoche Company, Nutley, NJ. Chemical synthesis of phosphorylated retinoids: Methods for the chemical synthesis of phosphorylated retinoids include the use of ditriethylamine phosphate as the phosphorylating agent in a mixture of acetonitrile and trichloroacetonitrile; the reaction is conducted in the dark and the phosphorylated retinoid is isolated and purified by chromatography on columns of DEAE-cellulose. (b) Selective extraction of retinyl phosphate and its glycosylated derivatives from membranes from different cell types: Retinyl phosphate and its glycosyl derivatives are extracted from the membranes with 99% methanol. This solvent leaves dolichyl phosphate and its glycosyl derivatives unextracted in the denatured membranes. (c) A solvent extraction procedure has also been developed to separate glycosyl derivatives of retinyl phosphate from glycosyl derivatives of dolichyl phosphate; the latter compounds remain in the lower phase of the Folch extraction procedure, whereas retinyl phosphate and its derivatives can be extracted in the upper phase. (d) An in vitro mouse epidermal cell system was used to study the activity of retinoids in preventing the expression of the neoplastic phenotype induced by phorbol esters. (e) An in vitro mouse epidermal cell culture system was used to study the biosynthesis of retinylphosphate and mannosylretinylphosphate.

Major Findings: Retinoids at 10^{-7} to 10^{-6} M were found to inhibit the expression of neoplastic transformation induced by phorbol esters in an in vitro mouse epidermal cell system. Retinoic acid and retinylphosphate were the most active compounds, whereas anhydroretinol, a compound with little growth-promoting activity, was the least active. Mouse epidermal cells cultured in the presence of retinyl acetate displayed the phenotype of a secreting epithelium. Retinyl acetate treated cultures incorporated labelled galactose, mannose and glucosamine into glycopeptides to a higher extent than their solvent treated controls; the stimulation of galactose incorporation was apparent within 2-3 hr from the addition of retinyl acetate to the culture medium. Retinyl acetate treatment did not alter significantly the level of [^{14}C]glucose in the culture medium, over a 24 hour period, nor did it affect the incorporation of labelled uridine into RNA.

Epidermal cells in culture synthesized retinylphosphate and doubly-labelled mannosylretinylphosphate in the presence of [15-^{3}H]retinol and [^{14}C]mannose. Cells incubated with [^{3}H or ^{14}C]labelled-galactose and [carbinol-^{14}C or ^{3}H]-labelled-retinol also synthesized a doubly-labelled galactolipid which had chromatographic properties of a glycosylretinylphosphate.

Radioactively labelled retinylphosphate and its glycosidic derivatives yielded anhydroretinol and the sugar phosphates as the major products of mild alkaline hydrolysis. The latter were hydrolyzed by strong acid and identified by gas liquid chromatography of the hexitol boronates.

These results demonstrate that retinyl acetate markedly alters glycoprotein synthesis in epidermal cells. It is further suggested that this alteration is mediated via the synthesis of retinylphosphate, mannosylretinylphosphate and a product with chromatographic properties of galactosylretinylphosphate. These biochemical reactions at the level of the cell membrane may explain the preventive action of retinoids in the promotion phase of carcinogenesis.

Significance to Biomedical Research and the Program of the Institute: This project contributes to the identification of key control mechanisms in the differentiation of epithelial cells; it is a part of the experimental pathology program addressed to the development of mechanisms of inhibition and control of epithelial carcinogenesis. The control of mucous-squamous cell differentiation and membrane growth is of value in understanding the mechanisms of carcinogenesis in epithelial tissues in general and in developing possible preventive measures. The vitamin controls epithelial cell differentiation and its abscence leads to hyperplasia of basal cells, squamous metaplasia and keratinization, a sequence of events similar to those taking place in several epithelia after exposure to carcinogens.

Proposed Course of Project: 1. To investigate the action of promoting agents such as phorbol esters on the biosynthesis of membrane glycoproteins and glycolipids. 2. To study the biochemical mechanisms by which retinoids inhibit the expression of the neoplastic phenotype in mouse epidermis.

Publications

Hassell, J. R., Silverman-Jones, C. S., and De Luca, L. M.: Stimulation of mannose incorporation into specific glycolipids and glycopeptides of rat liver by high doses of retinyl palmitate. J. Biol. Chem. 253: 1627-1631, 1978.

Sato, M., De Luca, L. M., and Muto, Y.: Effects of exogenous retinol and retinoic acid on the biosynthesis of [^{14}C]mannose labelled glycolipids and glycoproteins in rat liver. J. Nutr. Sci. Vitaminol. (TOKYO) 24: 9-23, 1978.

Sundaresan, P. R. and De Luca, L. M.: "Vitamin A contents of rat intestinal epithelium and jejunal mucinous adenocarcinoma. J. Natl. Cancer Inst. 58: 1643-1647, 1977.

Bergman, A., Mankowski, T., Chojnacki, T., De Luca, L. M., Peterson, E., and Dallner, G.: Glycosyl transfer from nucleotide sugars to C_{55}-, C_{85}-polyprenyl and retinylphosphates by microsomal subfractions and golgi membranes of rat liver. Biochem. J., 1978, In press.

De Luca. L. M.: The direct involvement of vitamin A in glycosyl transfer reactions of mammalian membranes. In Vitamins and Hormones. New York, Academic Press, 1977, pp. 1-57.

De Luca, L. M.: Vitamin A. In De Luca, H. F. (Ed.): Fat Soluble Vitamins. New York, Plenum, 1978, pp. 1-67.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 05004-01 EXP

PERIOD COVERED October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Carcinogen-Induced Lesions in Cultured Human Bronchus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Stoner	Expert	EXP, NCI
	C. Harris	Head, HTSS	EXP, NCI
	Y. Katoh	Visiting Fellow	EXP, NCI

COOPERATING UNITS (if any)

University of Maryland, School of Medicine, Baltimore, MD
Litton Bionetics, Inc., Kensington, MD

LAB/BRANCH
Experimental Pathology Branch

SECTION
Human Tissue Studies Section (HTSS)

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	0.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This is a new Project. Normal appearing human bronchial explants were exposed to chemical and physical carcinogens. Examination of the carcinogen-treated explants by light and electron microscopy revealed marked cellular atypia in the non-cornifying epidermoid metaplastic epithelium and in the exfoliated epithelial cells. In addition, autoradiograms showed an increase in labeling index with ^{3}H-Thymidine. The tumorigenic potential of these carcinogen-induced preneoplastic lesions is being studied in athymic nude mice.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-05004-01-EXP

Objectives: To demonstrate that environmental chemical carcinogens are capable of producing malignant changes in cultured human bronchial epithelial cells and to investigate the mechanism(s) of carcinogen-induced transformation.

Methods Employed: Normal human bronchial tissues are obtained from "immediate" autopsies. The tissue is maintained in culture as explants, isolated cells, or for xenotransplantation into athymic nude mice at a later date. The tissues are exposed to various doses of chemical carcinogens in vitro and/or as a xenograft. The morphological lesions induced in explants or in xenotransplants are examined by light and electron microscopy. The proliferative rate of bronchial epithelial cells in carcinogen-treated and control explants is examined by autoradiographic determination of the incorporation of ^{3}H-TdR into cell nuclei. The cultured cells are assayed for carcinogen-induced transformation by: a) determination of their ability to form colonies in soft agar; b) examination of exfoliated cells for evidence of cytological changes indicative of malignancy, and c) determining the ability of the cells to produce tumors after transplantation into athymic nude mice.

Major Findings: Human bronchial explant tissues have been exposed to either 7,12-dimethylbenz[a]anthracene (DMBA), amosite asbestos or combinations of DMBA and amosite asbestos, either as a single exposure or multiple exposures. The explants were taken either for light and electron microscopy and autoradiography or for xenotransplantation, 7, 14, and 21 days after the last exposure to the carcinogens. A single exposure to either amosite asbestos or amosite fibers coated with DMBA caused both a proliferative response as measured by ^{3}H-TdR labeled autoradiography and a multifocal noncornifying epidermoid metaplasia as measured by light and electron microscopy that persisted for at least 1, 2 and 4 weeks. In addition, the asbestos persisted within the epithelium and the stroma. Preneoplastic epithelial lesions induced by these carcinogens were characterized by loss of cell polarity, increased nuclear to cytoplasmic ratio, enlarged nucleoli and nuclear pleomorphism. Cytological examination of exfoliated cells from carcinogen-treated explants at 1, 2 and 4 weeks on coded slides revealed atypical cells including changes that were consistent with malignancy. Similar histological and cytological findings were found in bronchial explants exposed to repeated doses of benzo[a]pyrene-Fe_2O_3. The tumorigenic potential of carcinogen-exposed bronchial explants is being measured in athymic nude mice.

In addition, human bronchial explants have been exposed to N-methyl-N-nitro-N'-nitrosoguanidine (MNNG) or 4-nitroquinoline-1-oxide (4-NQO). The explants are then either harvested as above for microscopy and autoradiography or xenotransplanted into athymic nude mice.

Small segmental bronchi are being exposed to DMBA, aflatoxin B_1 or polonium 210 in athymic nude mice. The carcinogens are absorbed on lycra fibers and/or incorporated into pellets of beeswax and inserted into the lumen of the bronchial segments. The epithelial responses to these carcinogens are being investigated in serial-kill studies.

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MAY BE VIOLATED UP TO 2 OR 3 LETTERS.

Project No. Z01-CP-05004-01-EXP

Finally, attempts are being made to obtain transformed cells in culture from explant tissues treated in vitro with various carcinogens or from segmental bronchi exposed as xenografts to DMBA. The procedures being used for this purpose are essentially those described by Nettesheim and Marchok for hamster tracheal tissues. To date, no carcinogen-transformed cell lines have been established in culture.

Significance to Biomedical Research and the Program of the Institute: Carcinoma of the bronchus is among the leading causes of cancer death in the United States, and epidemiological studies indicate that environmental chemical carcinogens such as are found in tobacco smoke and in the atmosphere are the primary etiological agents of the disease. Therefore, it is important to develop models for the study of chemical carcingenesis in human bronchial tissues, including the demonstration of carcinogen-induced neoplasia. These model systems are of potential use for the: a) identification of environmental carcinogens for the bronchus; b) determining the role of cocarcinogens in the production of neoplasia in the bronchus, and c) investigating the mechanisms of carcinogen-induced neoplasia in bronchial tissues.

Proposed Course of Project: The future course of this project will include the following: 1) to continue investigation of the effects of single and multiple exposures of various doses of both chemical and physical carcinogens on the development of neoplastic lesions in explants, cultured epithelial cells and xenotransplants of human bronchial epithelium; 2) to determine whether promoting agents; e.g., 12-O-tetradecanoyl phorbol-13-acetate (TPA) will effect the development of carcinogen-induced lesions in cultured human bronchus; and 3) to develop methods for the early detection of carcinogen transformed cells in human bronchial tissues and cultured cells.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 05005-01 EXP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Carcinogenesis Studies in Human, Mouse and Rat Skin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: C. Harris, Head, HTSS — EXP, NCI

H. Autrup, Staff Fellow — EXP, NCI

COOPERATING UNITS (if any)

Pathological-Anatomical Institute, Copenhagen, Denmark

LAB/BRANCH

Experimental Pathology Branch

SECTION

Human Tissue Studies Section (HTSS)

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This is a new project. Methods have been developed to successfully transplant skin from humans, rats and mice onto athymic nude mice. Human skin has been transplanted from patients with and without xeroderma pigmentosa. The acute morphological effects of ultraviolet light, N-methyl-N'-nitro-N-nitrosoguanidine, 7,12-dimethylbenz[a]anthracene and 12-O-tetradecanoyl phorbol- 13-acetate have been determined. Based on these results chronic experiments are in progress.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-05005-01-EXP

Objectives: To develop a model system to study human skin carcinogenesis. To determine if species sensitivity (mouse, rat and human) to initiation-promotion is dependent on the skin and/or the host.

Methods: Xenotransplantation, light and electron microscopy, autoradiography.

Major Findings: A single exposure to either chemical or physical carcinogens caused a dose-dependent hyperplastic response in xenografted human skin. Cellular atypia and loss of cell polarity were found. Alterations in labeling index with ^{3}H-TdR are being measured.

Significance: Skin cancer is common in the human population. Studies of skin carcinogenesis in mice have contributed much to our understanding of the mechanism of initiation-promotion. Whether or not this phenomenon occurs in human tissues is unknown.

Proposed Course: Continuation of the studies outlined above.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 05006-01 EXP

PERIOD COVERED

October 1, 1977 to September 31, 1978

TITLE OF PROJECT (80 characters or less)

Identification of Normal and Neoplastic Human Epithelial Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Y. Katoh	Visiting Fellow	EXP, NCI
	G. Stoner	Expert	EXP, NCI
	C. Harris	Head, HTSS	EXP, NCI

COOPERATING UNITS (if any)

Univ. of Maryland, School of Medicine, Baltimore, MD; Laboratory of Immunodiagnosis, NCI, Bethesda, MD; Baylor College of Medicine, Houston, TX; University of Colorado, Boulder, CO

LAB/BRANCH

Experimental Pathology Branch

SECTION

Human Tissue Studies Section (HTSS)

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 0.75	PROFESSIONAL: 0.5	OTHER:

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Immunoperoxidase and immunofluorescence methods are being employed for the identification of normal and neoplastic human bronchial epithelial cells in tissue specimens and in primary cultures in vitro. Paraffin-embedded specimens of normal human bronchus from 20 patients were examined for the presence of: 1) A, B or H blood group antigens; 2) carcinoembryonic antigen (CEA); 3) alpha fetoprotein (AFP); 4) adrenocorticotropic hormone (ACTH); and 5) human chorionic gonadotrophin (HCG). A, B and H blood group antigens were localized in epithelial cells of the bronchial specimens and not in stromal fibroblasts. As expected, normal bronchial epithelial cells were negative for AFP, ACTH and HCG, although small amounts of CEA were found in the glycocalyx. Large amounts of CEA were detected in human lung tumors. Cultured bronchial epithelial cells also stained positively for A, B or H blood group antigens and fibroblast cells were negative. CEA, AFP, HCG and ACTH were not synthesized by normal bronchial epithelial cells. The distribution of tubulin in cultured

PHS-6040
(Rev. 10-76)

bronchial epithelial cells was different from that of fibroblasts. These methods will be useful for the identification of normal and carcinogen-transformed epithelial cells in bronchial explants, in exfoliative cytology and in cell cultures.

Objectives: To use immunoperoxidase and immunofluorescence methods for: a) the identification of normal and tumor cells in paraffin-embedded tissues of human bronchus; b) the detection of normal and carcinogen-transformed human bronchial epithelial cells in explants and in cell cultures; c) the determination of the effects of chemical carcinogens on components of the cytoskeleton; and d) to correlate the above with the ultrastructure of human cells as determined by high-voltage electron microscopy.

Methods Employed: Normal and tumorous human bronchial specimens are obtained at either surgery or "immediate" autopsy, fixed and embedded in either paraffin or plastic. The paraffin blocks were sectioned and placed on slides which were smeared with glue as adhesive. The paraffin sections of human bronchus were digested with trypsin because this treatment has been reported to decrease the non-specific immunofluorescent staining and unmask immunoreactive sites of certain antigens. The explants of human bronchus also were cultured on glass coverslips in plastic dishes. The cell outgrowth from the explant on the coverslip was fixed with cold acetone for staining. For detection of A,B and H blood group antigens, HCG and ACTH, double-bridge immunoperoxidase and immunofluorescent techniques were used. For CEA and AFP detection, the triple-bridge "peroxidase-anti-peroxidase (PAP)" technique was used. Bronchial mucosa cells are also examined by low- and high-voltage electron microscopy.

Major Findings: Treatment of the bronchial specimens with trypsin markedly increased the immunoperoxidase and immunofluorescent staining reactions for the blood group antigens. The specimens were exposed to trypsin for periods ranging from 15 minutes up to 4 hours; treatment for 2 hours was sufficient for optimal staining. The staining reaction was strongly positive in all specimens taken from patients with blood groups A, B or AB but not with blood group H. Staining for the blood group antigens was localized specifically in the epithelium and mucous glands. Mesenchymal cells were not stained.

AFP, HCG and ACTH were all negative in specimens of normal human bronchi and were positive in tumors, pituitary and/or placenta. However, after trypsinization, CEA was weakly positive in normal bronchial specimens.

When the cells on glass coverslips were stained for A, B, and H blood group antigens, the specific blood group antigens were localized only in the polyhedral (epithelial-like) and not in the fusiform (fibroblast-like) cells. These results suggest that polyhedral cells which stained with blood group antigens were derived from the epithelium of human bronchus and the fusiform cells were fibroblasts which originated from the stroma. CEA, AFP, HCG and

ACTH were all negative in both polyhedral cells and in the fusiform cells of the outgrowth.

When the outgrowth was stained with antibody to tubulin, the distribution of tubulin in the polyhedral cells was different from that of the fusiform cells. Examination of the cells by electron microscopy is in progress.

Significance to Biomedical Research and to the Program of the Institute: An important aspect of in vitro carcinogenesis studies is the development of methods for the identification of normal and carcinogen transformed epithelial cells. The two major goals in these studies are: a) the identification and separation of epithelial cells from fibroblast cells, and b) the detection of transformed cells in carcinogen-treated epithelial cell populations. The blood group antigens appear to be useful markers for normal epithelial cells of a variety of human tissues, including the lung. In addition, other workers have reported that the blood group antigens are not found in tumors of several human tissues. AFP, CEA, ACTH and HCG are produced by certain lung tumors and cultured lung tumor cells; therefore, the expression of one or more of these antigens may be useful for the detection of carcinogen-induced cell transformation. Finally, some or all of these antigens might also be used for the identification of normal and neoplastic cells in other human target tissues; e.g., colon, liver, and pancreatic duct.

Proposed Course of Project: To use immunoperoxidase and immunofluorescence methods for the detection of the above-described antigens in paraffin- and plastic-embedded sections of human lung tumors and in normal and carcinogen-treated bronchial explants and in cultured cells. Other antigens will also be investigated as potential markers of epithelial cells; e.g., type IV collagen, β-lymphocyte antigens, and surface membrane antigens of bronchial epithelial cells prepared by injection of rabbits with bronchial cell preparations. The distribution of components of the cell cytoskeleton; microfilaments (actin) and microtubules (tubulin) in normal and carcinogen-treated human bronchial tissues will also be investigated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 05007-01 EXP

PERIOD COVERED October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Mechanism of mutagenesis and sister chromatid exchanges in Chinese hamster V79 cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	I. C. Hsu	Research Scientist	EXP, NCI
Other:	T. Bowden	Staff Fellow	EXP, NCI
	M. Bradley	Staff Fellow	LMPH, NCI
	C. Harris	Head, HTSS	EXP, NCI

COOPERATING UNITS (if any)

LAB/BRANCH Experimental Pathology Branch

SECTION Human Tissue Studies Section

INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.25	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The biological significance of sister chromatid exchanges (SCE) and their relationship to mutagenesis is being investigated. DNA postreplication repair, SCE, DNA breaks and mutagenesis were determined in Chinese hamster V79 cells treated with direct-acting chemical and physical mutagens. Dose-response curves showed that benzo[a]pyrene diol-epoxide-I was more potent as a mutagen and also produced a higher frequency of SCE in V79 cells than did benzo[a]-pyrene diol-epoxide-II. However, cis- and trans-diaminodichloro-P (II) produced the same frequency of SCE in V79 cells, but only the cis compound was mutagenic. This suggests that SCE may not be directly related to mutagenesis. Caffeine alone does not: 1) alter the frequency of SCE; 2) inhibit DNA postreplication repair; 3) cause DNA breaks; or 4) cause an increase in mutations of V79 cells. When V79 cells were treated with both benzo[a]pyrene diol-epoxide-I and caffeine, the cytotoxic effects were synergistic and DNA postreplication repair was inhibited. However, synergistic effects on either frequency of SCE or frequency of mutation were not detected.

PHS-6040
(Rev. 10-76)

Objectives: To determine the biological significance of sister chromatid exchanges, its mechanism and relationship to DNA damage and to mutagenesis in Chinese hamster V79 cells.

Methods Employed: Treatment of V79 cells with several different types of direct-acting chemical and physical mutagens; selection of the mutants by medium containing ouabain; alkaline sucrose gradient centrifugation for the determination of DNA postreplication repair; DNA elution; fluorescent plus Giemsa chromosome stain to determine sister chromatid exchanges.

Major Findings: Both *cis*- and *trans*-diaminodichloro-P (II) increase the frequency of SCE in V79 cells but only the *cis* compound is mutagenic indicating that increase of SCE may not be associated with mutation frequency; hydrogen peroxide also causes SCE and is not mutagenic. Caffeine and diol-epoxide-I are cytotoxic and inhibit DNA postreplication repair in a synergistic manner but SCE and mutation frequency are not altered by caffeine. This suggests that delay in post-replication DNA repair does not necessarily increase error-prone DNA synthesis.

Significance to Biomedical Research and the Program of the Institute: Several methods are used for screening chemical carcinogens. To evaluate the validity of the tests or the significance of the results, it will be helpful to understand the mechanism of the test and its relation to carcinogenesis. These studies of DNA damage, DNA repair synthesis, and sister chromatid exchange will provide information on the mechanism of mutagenesis.

Proposed Course of Project: To determine the biochemical and functional interactions between different classes of chemical and physical mutagens (carcinogens) and DNA.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 05008-01 EXP

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Explant and Cell Culture of Human Pulmonary Tissue

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. Stoner	Expert	EXP, NCI
	C. Harris	Head, HTSS	EXP, NCI
Other:	H. Autrup	Staff Fellow	EXP, NCI

COOPERATING UNITS (if any)

University of Maryland, School of Medicine, Baltimore, MD
Litton Bionetics Inc., Kensington, MD

LAB/BRANCH

Experimental Pathology Branch

SECTION

Human Tissue Studies Section (HTSS)

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.75	0.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Model systems for the study of carcinogenesis in cultured human bronchus, peripheral lung, and pulmonary alveolar macrophages have been developed. Explants of human bronchus and peripheral lung have been maintained in culture for 4 months and 24 days, respectively. The metabolism of several classes of chemical carcinogens has been investigated in human bronchus, and that of benzo[a]pyrene in peripheral lung and alveolar macrophages. Human bronchial epithelial cells have been grown and maintained in culture for periods of 3-4 months. Attempts are being made to improve the culture conditions for these cells. It is anticipated that these model systems will be useful for: 1) identification of environmental carcinogens for human lung; 2) determination of the metabolic pathways for carcinogens in these tissues; 3) identifying host factors determining susceptibility to chemically induced lung cancer; and 4) evaluating new methods of prophylactic intervention in populations at high risk of developing lung cancer.

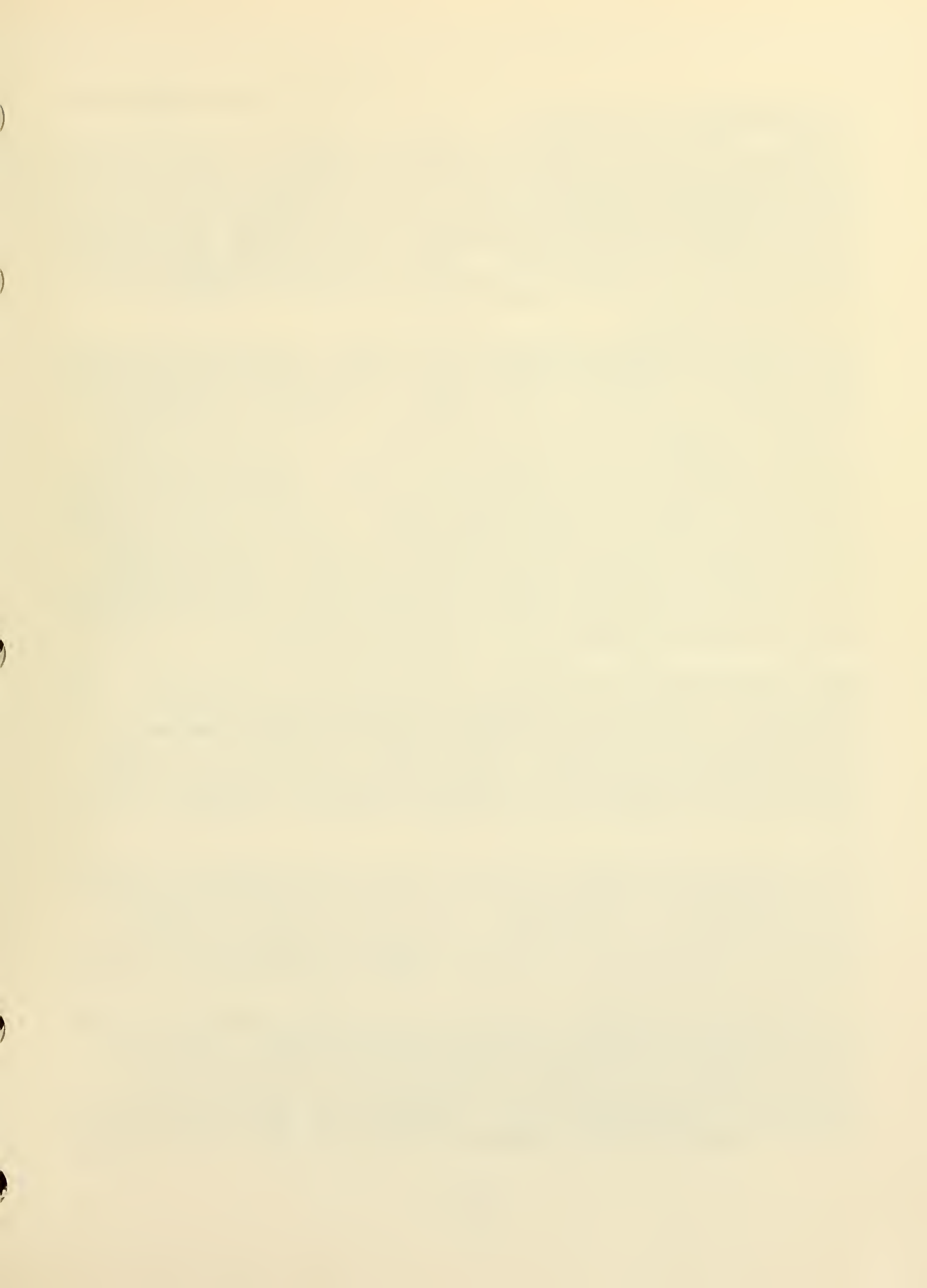
PHS-6040
(Rev. 10-76)

Objectives: To develop model systems to study chemical carcinogenesis in human lung tissues. These involve 2 major facets: 1) obtainment of viable human lung; and 2) maintenance of epithelial cell types in lung tissue in explant and cell cultures.

Methods Employed: Human lung tissues are obtained at either surgery or "immediate" autopsy. The explants are maintained in an organ culture chamber under controlled environmental conditions. The viability of the explants is assessed by: 1) high-resolution light microscopy and electron microscopy, and 2) radioautographic techniques using labelled precursors for DNA, RNA and protein. Cultures of pulmonary alveolar macrophages are obtained by trypsinizing human peripheral lung tissues, inoculating the cells into tissue culture dishes and removing the nonadherent cells after 21/2-3 hours. More than 95% of the adherent cells after this period are alveolar macrophages. Cultures of normal human bronchial epithelial cells are being established for studies of in vitro chemical carcinogenesis. During culture of the bronchial explants, cells with a polyhedral morphology (epithelial-like) and with a fusiform morphology (fibroblast-like) migrate from the outer edge of the explant onto the culture dish. By serial transfer, it is possible to obtain 3-4 outgrowths from a single explant. The effects of putrescine and of various culture media and sera on the nature and degree of cellular outgrowth from the bronchial explants have been investigated. In addition, the effects of various enzymes and chelating agents on disaggregation of the cells to obtain secondary cultures have been studied. Finally, the ability of the epithelial-like cells to form colonies from single cells under various conditions has been determined. Attempts are being made to identify the cells by; a) electron and light microscopy; b) cytochemical techniques, and c) immunofluorescent and immunoperoxidase techniques using antibody prepared to a variety of antigens associated with both normal and tumorous human bronchial epithelial cells.

Major Findings: Human bronchi can be cultured in vitro for at least 4 weeks in chemically defined CMRL-1066 medium supplemented with insulin (1.0 μg/ml), hydrocortisone hemisuccinate (0.1 μg/ml), beta-retinyl acetate (0.1 μg/ml), 2 mM L-glutamine, penicillin G (100 units/ml), and streptomycin (100 μg/ml), and for at least 16 weeks in medium supplemented with 5% heat-inactivated fetal calf serum. During the first 4 weeks in sèrum-supplemented medium, the bronchial epithelial cells retain essentially normal morphology and actively incorporate precursors into DNA, RNA and protein. After culture for more than eight weeks, the epithelium becomes progressively more hyperplastic and is composed principally of small mucous granule cells and basal cells with few ciliated cells and mucous cells. Mucus production decreases with increasing time of explant incubation.

During culture, cells with a polyhedral morphology (epithelial-like) and with a fusiform morphology (fibroblast-like) migrate from the outer edge of the explants onto the culture dish. By serial transfer of the explants, it is possible to obtain 3-4 outgrowths of polyhedral cells; in subsequent transfers, the outgrowth is composed principally of fusiform cells. The degree of



pronase results in the destruction or irreversible damage of the majority of the cells. However, a gentle method of dissociating the cells involves treatment of the cells with a balanced salt solution containing a high level of potassium followed by a second solution containing 0.2% EGTA. Using this method of dissociation, secondary and tertiary cultures composed of only polyhedral cells have been obtained from primary cultures grown in the presence of 10 mM putrescine have been obtained. When plated at low density as single cells, polyhedral cells will not form colonies when grown in regular or "conditioned" medium, in a feeder layer of irradiated human lung fibroblasts, or on collagen substrates.

Explants of human peripheral lung tissues have been maintained in culture on gelatin sponge for a period of 24 days. During the incubation, there was incorporation of ^{3}H-thymidine into the nuclei of alveolar type II epithelial cells and bronchial epithelial cells. Therefore, two potential target cells for tumors in peripheral lung have been maintained in culture for prolonged periods. The metabolism of benzo[a]pyrene (BP) in human peripheral lung explants cultured for up to 7 days was investigated. Human lung explants had measurable aryl hydrocarbon hydroxylase activity and could metabolize BP into forms that were bound to cellular DNA and protein. Peripheral lung had significantly lower aryl hydrocarbon hydroxylase activity than cultured bronchus but both tissues had similar binding levels of BP to DNA. Radioautographic studies indicated that all cell types in the peripheral lung can metabolize BP. The major ethylacetate extractable metabolites of BP formed by peripheral lung were tetrols and trans-7,8-diol. The primary water soluble metabolite released with arylsulfatase and β-glucuronidase was 3-OH-BP.

Significance to Biomedical Research and to the Program of the Institute: The extrapolation of experimental animal data to man is a major problem in carcinogenesis. One approach to provide a link between these experimental data and human cancer is to develop model systems in cultured human tissues for carcinogenesis investigations. Such systems could be used for the identification of carcinogens and their metabolic pathways and ultimately, in the identification of individuals who are highly susceptible to chemical carcinogens.

Current results indicate that explants of human bronchus and peripheral lung can be maintained for prolonged periods in culture. The metabolism of chemical carcinogens can be investigated in these tissues as well as studies of carcinogen-mediated cell transformation. Monolayer cultures of bronchial epithelial cells can also be used for carcinogenesis studies and should permit large scale investigations of a variety of environmental chemicals.

Proposed Course of the Project: Attempts are being made to improve each facet of this program. Conditions for organ culture of human bronchi are being modified to prevent the hyperplasia associated with prolonged culture. Considerable progress has been made in the identification of human bronchial epithelial cells in culture using such methods as: a) light and electron microscopy, b) cytochemistry, and c) the detection by immunofluorescence and immunoperoxidase techniques of antigens associated with normal and carcinogen

transformed cells. In addition, research is underway to define the optimum growth conditions of bronchial epithelial cells; i.e., substrate, temperature, nutritional and atmospheric conditions. The methodologies developed from growing bronchial epithelial cells in vitro might also be applicable to the culture of other important human target epithelial cells; e.g., pancreatic duct cells and colon cells.

Publications

Stoner, G. D., Harris, C. C., Autrup, H., Trump, B. F., Kingsbury, E. W., and Myers, G. A.: Explant culture of human peripheral lung. I. Metabolism of benzo[a]pyrene. Lab. Invest., 1978, In press.

SUMMARY REPORT

LUNG CANCER BRANCH

October 1, 1977 through September 30, 1978

"Plans, develops and implements a research program on the pathogenesis and prevention of lung cancer involving (1) identification and study of agents and mechanisms involved in the induction and development of lung cancer in man; (2) correlation of findings in human and animal studies in order to define relevance of animal models for study of human lung cancer; and (3) identification and study of agents and mechanisms capable of preventing the development of lung cancer. All these studies are pursued in the whole organism, in isolated cellular systems involving organ and tissue culture, and at the molecular level, in animals and man."

Current studies in the Lung Cancer Branch include investigations on the carcinogenic effects of different chemicals on the respiratory epithelium in the whole animal, the use of short-term and long-term organ culture systems to study the effects of carcinogens and anti-carcinogens on respiratory epithelium, and investigation of the cellular and molecular mechanism of action of the anti-carcinogenic family of compounds known as retinoids, comprised of vitamin A and its synthetic analogs. Studies are pursued within the Branch in a manner that allows correlation of findings at the molecular, cellular, and whole animal level. The following is a summary of the investigations within the Branch:

Lung Cancer Pathogenesis Section - *"(1) Develops and evaluates animal models for study of induction and prevention of lung cancer, and the role of various chemicals in inducing or preventing lung cancer. Studies involve the administration of carcinogenic and anti-carcinogenic agents to experimental animals and the evaluation of their effects on the development of lung cancer; (2) develops and evaluates organ culture and tissue culture methods for study of induction and prevention of lung cancer. Studies measure the effects of carcinogenic and anti-carcinogenic agents on the cell population which is directly involved in the development of lung cancer; and (3) studies molecular processes involved in control of cellular differentiation in respiratory epithelium as related to the induction and prevention of lung cancer. Particular emphasis is placed on the biochemical mechanisms whereby selected compounds inhibit or prevent the development of lung cancer."*

(1) Animal Studies of Respiratory Carcinogenesis - A new technique for study of respiratory carcinogenesis, employing a special cannula, has been developed very recently at the Oak Ridge National Laboratory. This cannula allows direct application of soluble carcinogens to a particular segment of the respiratory tract of an experimental animal. This particular area then can be observed for the development of preneoplastic and neoplastic lesions, and factors that enhance or prevent the development of respiratory cancer can be intensively studied. The advantage of the new method is that one can study the pathogenesis of respiratory cancer at a defined locus in the respiratory tract, in an otherwise healthy animal that does not have

multiple lesions throughout both lungs. Within the past year, extensive studies with this special cannula have been pursued in the Lung Cancer Branch. Hamsters have been exposed to various levels of nitrosomethylurea with this cannula, and squamous cell cancer has been induced. During the course of carcinogenesis, cytology specimens have been obtained without sacrificing the hamsters and have been evaluated with exfoliative cytological techniques. This will provide a useful method for further studies of markers of premalignant states.

During the past year, an image analyzing microscope has been used to quantitate some of the preneoplastic changes that occur in respiratory epithelium. By use of this machine, changes in nuclear and cytoplasmic sizes have been measured in cytology samples collected from tracheas exposed to carcinogens, which enable these samples to be distinguished from those that were unexposed.

(2) Organ Culture Studies on the Isolated Tracheobronchial Epithelium of the Hamster - Excellent progress has been made in this area during the past 12 months. Extensive use is now being made of the system which has been developed in the Lung Cancer Branch for long term organ culture of hamster trachea. A chemically defined medium, supplemented with insulin and hydrocortisone, is being used to give excellent long-term in vitro survival of hamster tracheobronchial epithelium, for 2 weeks or more. Using this system, it has been demonstrated that numerous synthetic analogs of vitamin A (retinoids) can reverse keratinizing, squamous metaplastic lesions of hamster tracheal epithelium. In addition to the further confirmation of previous findings that analogs with modifications in the ring portion of the molecule are highly active biologically, it has been found that the polar, terminal portion of the molecule can also be modified with substantial retention of activity. These new synthetic molecules include 4-hydroxyphenyl retinamide, retinylidene acetylacetone, and 2-retinylidene-1,3-cyclohexanedione, which appear to be substantially less toxic than natural forms of vitamin A. The reduction in toxicity offers major advantages in terms of development of a synthetic analog which would eventually be useful for prevention of epithelial cancer in man. These analogs are also being evaluated for in vivo effects on specific target epithelia, such as respiratory epithelium, bladder epithelium, or mammary epithelium. The scientific collaboration of Hoffmann-La Roche Inc., Johnson & Johnson, and BASF Aktiengesellschaft has been invaluable in the performance of the above studies, as has the cooperation of the Medicinal Chemistry Section, Laboratory of Chemistry, NIAMDD.

In biochemical studies using hamster tracheal organ cultures, all-trans-[11,12-^{3}H]retinoic acid has been shown to be converted to several more polar metabolites in hamster tracheal organ cultures. Several of these metabolites formed in vitro cochromatograph in two high-pressure liquid chromatography systems with compounds found in vivo in the hamster intestine and urine. This suggests that the in vitro system is generating physiologically significant material. Using a hamster tracheal organ culture assay, one of these metabolites has been shown to possess activity in reversing the keratinization process found in retinoid-deficient trachea. This biologically active compound is capable of being metabolized

by the trachea in vitro to a biologically inactive compound. In order to generate a sufficient quantity of this biologically active compound for structure determination, a liver generating system has been set up that is capable of producing compounds that comigrate with the tracheal metabolites in several high-pressure liquid chromatographic systems. Using this liver generating system, the biologically active metabolite is currently being isolated in a sufficient quantity for mass spectral and nuclear magnetic resonance determinations.

(3) Pharmacology and Biochemistry of Control of Epithelial Cell Differentiation with Retinoids - Since the possible eventual use of retinoids to prevent cancer in man is dependent on further understanding of the pharmacological properties of these agents, a new program has been established in the Branch to develop basic knowledge in this area, in which relatively little is known. New staff have been recruited during the past year and new methods are being developed. In particular, the new technique of high pressure liquid chromatography is being applied to investigate the metabolism and tissue distribution of retinoids. Initial efforts have been largely devoted to development of analytical methods. One area that has already received special attention is the problem of toxicity of retinoids, which limits the potential applications of some of these agents. It has already been found that the presence of a terminal carboxylic acid group in a retinoid molecule is often associated with highly toxic properties. Studies pursued in the Branch during the past year have led to the conclusion that alteration of the acid function to a less polar derivative (such as an ether, an amide, or a hydrocarbon) may have the very desirable effect of lessening the toxic properties of the molecule, without appreciably diminishing the desired effects on cell differentiation. Moreover, it has been found that the pattern of tissue distribution of retinoids is markedly changed by alteration of the polar end group. This has the very practical result of "targeting" the retinoid to specific organs, such as lung, bladder, or breast, in which it is desired to prevent cancer.

A particularly important finding during the past year has been the identification of a new class of less toxic retinoids, namely the retinylidene-1,3-diketones. These compounds have been found to have very high potency in vitro, to be much less toxic than retinyl acetate or retinoic acid, and to have increased tissue distribution to fatty organs such as the breast. They are currently being evaluated for prevention of mammary cancer in experimental animals.

In biochemical studies, in vitro metabolism of retinoic acid has been found to occur in NADPH and O_2 supplemented 10,000 x g fraction from various tissues of vitamin A deficient hamsters. The in vitro metabolism parallels in part that found in vivo. The in vivo and the in vitro metabolism in some tissues are highly responsive to in vivo induction with retinoids but not with other common inducers such as phenobarbital. Other tissues are insensitive to this induction. The ability of a series of synthetic retinoids to induce the metabolism of retinoic acid appears to parallel their ability to be converted to retinoic acid in vivo. Three major metabolites isolated from this in vitro system have each been found to have biological activity in a tracheal organ culture assay system, and attempts at their identification are currently underway.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO-1-CP-04494-02-LC

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Metabolism and Tissue Distribution Studies of Natural and Synthetic Retinoids

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Anita B. Roberts Staff Fellow LC NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Lung Cancer Branch

SECTION
Lung Cancer Pathogenesis Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to increase our understanding of the metabolism of retinoids. Such information must be had before a rational approach can be taken to the design of new retinoids with greater activity in the reversal of preneoplasia in epithelial tissues. The investigation is along two basic lines: 1) The study of the in vitro metabolism of retinoic acid and synthetic retinoids in subcellular systems of target tissues as compared to non-target tissues, and 2) the study of the biochemical activation mechanisms of the synthetic retinoids.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04494-02-LC

Project Description

Objectives: The purpose of this study is to investigate the metabolism of retinoids and from this to gain insight into the particular chemical modifications of synthetic retinoids which might enhance activity, lower toxicity, or change the tissue distribution of the compound. Part of this study is aimed at distinguishing between "activating" metabolic conversions and "deactivating" reactions which earmark a compound for elimination. It is hoped that the development of an *in vitro* system for retinoid metabolism will facilitate both identification of metabolic products and the comparison of retinoid metabolism in target versus non-target organs.

Methods Employed: An *in vitro* system for studying retinoid metabolism has been developed using a 10,000 x *g* supernatant fraction of hamster intestinal mucosa, liver, testis, and kidney. Ultracentrifugation is used to isolate subcellular fractions. Radioactive retinoids are used as substates.

The techniques employed for the detection of metabolites include freeze-drying, extraction, separation by high-pressure liquid chromatography (HPLC), and liquid scintillation counting.

Techniques for the study of the *in vivo* disposition of various retinoids involve an adaptation of a previously used extraction procedure to final analysis on HPLC.

Major Findings: *In vitro* metabolism of retinoic acid has been found to occur in NADPH and O_2 supplemented 10,000 x *g* fraction from various tissues of vitamin A deficient hamsters. The *in vitro* metabolism parallels in part that found *in vivo*. The *in vivo* and the *in vitro* metabolism in some tissues are highly responsive to *in vivo* induction with retinoids but not with other common inducers such as phenobarbital. Other tissues are insensitive to this induction. The ability of a series of synthetic retinoids to induce the metabolism of retinoic acid appears to parallel their ability to be converted to retinoic acid *in vivo*. Three major metabolites isolated from this *in vitro* system have each been found to have biological activity in a tracheal organ culture assay system, and attempts at their identification are currently underway.

Significance to Biomedical Research and the Program of the Institute: A greater understanding of the metabolism of retinoids with respect to the identification of the reactive sites on the molecule, as well as the particular metabolic characteristics of the various target tissues should greatly influence the design of new retinoids with better properties than the natural vitamin A compounds for the chemoprevention of preneoplasia of epithelial tissues.

Proposed Course of Project: Future work on this project will involve 1) continued investigation of the *in vitro* metabolism of retinoic acid and synthetic retinoids in both "target" and "non-target" tissues, 2) relating the *in vitro* studies to the *in vivo* metabolism, 3) identification of the *in vitro* metabolites of retinoic acid from hamster liver and intestinal systems

(a joint effort with C. A. Frolik of our laboratory), and 4) investigation of the mechanism whereby synthetic retinoids enter the scheme of activation to biologically active intermediates.

Publications

Newton, D.L., Frolik, C.A., Roberts, A.B., Smith, J.M., Sporn, M.B., Nürrenbach, A., and Paust, J.: Biological activity and metabolism of the retinoid, axerophthene (vitamin A hydrocarbon). _Cancer Res._, in Press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04549-06-LC

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Organ Culture Studies on Tracheobronchial Epithelium

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Michael B. Sporn   Chief, Lung Cancer Branch   LC NCI

COOPERATING UNITS (if any)

Medicinal Chemistry Section, Laboratory of Chemistry, NIAMDD

LAB/BRANCH
Lung Cancer Branch, Carcinogenesis Research Program

SECTION
Lung Cancer Pathogenesis Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	1.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS   ☐ (b) HUMAN TISSUES   ☒ (c) NEITHER

☐ (a1) MINORS   ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The ability of vitamin A and its synthetic analogs (retinoids) to reverse a potentially premalignant lesion of tracheobronchial epithelium is being studied in organ culture. Keratinized, squamous metaplastic lesions are established in organ cultures of hamster tracheas. Addition of active retinoids causes reversal of these lesions and restoration of healthy mucociliary epithelium. Several new retinoids have been found to be extremely active and relatively free of toxicity, using this experimental system.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: The principal objective is to study the direct effects of carcinogens and anti-carcinogenic agents (such as retinoids) on the cell population which is of immediate relevance for development of the most common form of human lung cancer, namely bronchogenic carcinoma. The principal experimental system that is being used measures the reversal of a potentially premalignant lesion, keratinized squamous metaplasia, by the addition of vitamin A and its synthetic analogs (retinoids).

Methods Employed: Classical methods of organ culture are being used. Tracheas are being obtained from either normal animals, from vitamin A-deficient animals, or from animals treated with respiratory carcinogens. After lesions have become established in organ culture, the cultures are treated with different retinoids. The restoration of a normal ciliated and mucus-producing epithelium is then measured.

Major Findings: The optimal conditions for long-term organ culture of tracheobronchial epithelium of vitamin A-deficient hamsters have previously been defined, using medium CMRL-1066.

New synthetic retinoids, such as 4-hydroxyphenyl retinamide, retinylidene acetylacetone, and 2-retinylidene-1,3-cyclohexanedione have been found to be highly active compounds for reversal of keratinized squamous metaplasia. All of these compounds have been found to be significantly less toxic than the natural retinoid, retinyl acetate.

Significance to Biomedical Research and the Program of the Institute: The finding that new analogs of retinyl acetate, with modifications in the side chain and terminal portion of the molecule, are directly active on the tracheal epithelium is particularly important. This finding opens the way for synthesis of new forms of vitamin A that may have greater pharmacological usefulness in man for prevention of lung cancer. Studies are currently in progress in the Lung Cancer Branch to evaluate the direct effects of synthetic retinoids on preneoplastic respiratory epithelium of hamsters.

Proposed Course of Project: To continue as outlined above.

Publications

Sporn, M.B.: Prevention of Epithelial Cancer by Vitamin A and Its Synthetic Analogs (Retinoids). In: Origins of Human Cancer. New York, Cold Spring Harbor Laboratory, 1977, pp. 801-807.

Sporn, M.B.: Vitamin A and its Analogs (Retinoids) in Cancer Prevention. In: Winick, M. (Ed.) Nutrition and Cancer. New York, John Wiley & Sons, Inc., 1977, pp. 119-130.

Sporn, M.B., Newton, D.L., Smith, J.M., Acton, N., Jacobson, A.R., and Brossi, A.: Retinoids and cancer prevention: The importance of the terminal group of the retinoid molecule in modifying activity and toxicity. (Eds.) A. C. Griffin and C. R. Shaw. New York, Raven Press, 1978, in Press.

Newton, D.L., Frolik, C.A., Roberts, A.B., Smith, J.M., Sporn, M.B., Nürrenbach, A., and Paust. J.: Biological activity and metabolism of the retinoid axerophthene (vitamin A hydrocarbon). Cancer Res., in Press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04574-02-LC

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

The effect of retinoids on carcinogenesis in the lung

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Sherman F. Stinson   Staff Fellow   LC NCI

COOPERATING UNITS (if any)

none

LAB/BRANCH

Lung Cancer Branch, Carcinogenesis Research Program

SECTION

Lung Cancer Pathogenesis Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS   ☐ (b) HUMAN TISSUES   ☒ (c) NEITHER

☐ (a1) MINORS   ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the purpose of this study to define the effects of retinoids on carcinogenesis in the tracheal epithelium. To achieve this goal, several areas are being simultaneously investigated. 1) A reproducible model for the study of lung cancer using N-methyl-N-nitrosourea (NMU) or other water soluble carcinogens, in the hamster trachea is being developed. 2) Various retinoids are being tested, using this model, for their ability to modulate the effects of administered carcinogens. 3) Methods for collecting exfoliated cells from the lung are being investigated.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04574-02-LC

Project Description

Objectives: The objectives of this project are to 1) develop a reproducible experimental model for the study of carcinogenesis in the respiratory tract, and 2) determine the effects of various retinoids on respiratory carcinogenesis.

Methods Employed: Using a specially designed intra-tracheal catheter, the dose-response relationship of hamster trachea to NMU has been investigated. In addition, serial sacrifice studies to more clearly define the pathogenesis of preneoplastic and neoplastic lesions in this system have been conducted. These changes were correlated with changes in exfoliated cells from the respiratory tract.

Two groups of hamsters being fed maximally tolerated doses of 13-cis-retinoic acid have been treated with NMU, as described above, to determine the effect of this retinoid on tracheal carcinogenesis.

Major Findings: Analysis of the studies described above are not yet completed. Preliminary evaluations, however, have indicated that 10-15 weekly treatments with a 0.5 % solution of NMU results in a 50 - 75 % tumor incidence by 25 weeks with minimal mortality due to treatment-related toxicity. A dose response relationship is found with varying both the concentration of NMU and the number of exposures, but doses above those described result in treatment-related toxicity.

The neoplasms are squamous or mixed squamous-glandular, and invasive. The pathogenesis of the lesions is being analyzed.

Under the conditions of these experiments, no protective effect of 13-cis-retinoic acid against tumor induction has been observed, although high mortality due to NMU toxicity made interpretation of results difficult in the 2 retinoid studies.

Significance to Biomedical Research and the Program of the Institute: These studies have significance in two major areas: The development of an animal model system for the study of lung cancer; the definition of the effects of retionoids on preneoplasia in the lung. The use of the new cannula system for intratracheal instillation of carcinogens is particularly important, since it allows direct application of carcinogen to a well-defined segment of the respiratory tract. This particular area then can be observed for the development of prenoplastic and neoplastic lesions, and factors that prevent the development of respiratory cancer can be intensively studied.

Proposed Course of Project: Further studies are in progress and proposed to test the effects of 13-cis-retinoic acid, and other retinoids, on this system using different dose levels of carcinogen. Experiments are also proposed to to investigate the possibility of using alternative water-soluble carcinogens, with lower toxicity, in the system.

Publications

Stinson, S.F., Lilga, J.C., and Sporn, M.B.: Automated image analysis of nuclear and cytoplasmic changes in exfoliated cells from tracheas treated with carcinogen. Proc. Electron Micros. Soc Am. 35:220-221, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04584-03-LC

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Control of Epithelial Cell Differentiation and Carcinogenesis by Vitamin A.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: C.A. Frolik Senior Staff Fellow LC NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Lung Cancer Branch, Carcingenesis Research Program

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The ultimate goal of this project is to find an agent that will cause a reversal of preneoplasia in epithelial tissue. Currently, the compounds being investigated are vitamin A and its analogs (retinoids). Emphasis has been placed on the metabolism of a natural retinoid, retinoic acid. It has been demonstrated that a hamster tracheal organ culture system will metabolize retinoic acid to more polar compounds that cochromatograph in several reverse phase high-pressure liquid chromatography systems with metabolites found in vivo in the hamster intestine and urine. A knowledge of the structure and the biological activity of the various naturally occurring metabolites of retinoic acid will help to decipher the biochemical mechanism of action of vitamin A in determining epithelial cell differentiation. It will also aid in designing analogs of vitamin A that will display biological activity in the reversal of preneoplasia and, at the same time, will show low toxicity in the animal.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: It is the purpose of this study to determine the role of vitamin A in the control of epithelial cell differentiation - both normal and carcinogenic. Special emphasis is being placed on a comparison of the metabolism and biological activity of the natural retinoids with the various vitamin A analogs in an effort to find a compound displaying high activity in the reversal of preneoplasia while also showing low levels of toxicity.

Methods Employed: Initial experiments have concentrated on the metabolism of all-*trans*-retinoic acid using a hamster tracheal organ culture system. This system has been shown to be responsive to nanomolar concentrations of retinoic acid in causing the reversal of the process of keratinization caused by retinoid-deficiency. The metabolites synthesized *in vitro* are extracted from the culture medium utilizing a lyophilization method. They are then divided into more polar and less polar fractions by a butanol extraction procedure and are finally separated by reverse phase high-pressure liquid chromatography. The final pure metabolites will be identified using mass spectroscopy and nuclear magnetic resonance.

Major Findings: All-*trans*-[11,12-^{3}H]-retinoic acid has been shown to be converted to several more polar metabolites in hamster tracheal organ cultures. Several of these metabolites formed *in vitro* cochromatograph in two high-pressure liquid chromatography systems with compounds found *in vivo* in the hamster intestine and urine. This suggests that the *in vitro* system is generating physiologically significant material. Using a hamster tracheal organ culture assay, one of these metabolites has been shown to possess activity in reversing the keratinization process found in retinoid-deficient trachea. This biologically active compound is capable of being metabolized by the trachea *in vitro* to a biologically inactive compound. In order to generate a sufficient quantity of this biologically active compound for structure determination, a liver generating system has been set up that is capable of producing compounds that comigrate with the tracheal metabolites in several high-pressure liquid chromatographic systems. Using this liver generating system, the biologically active metabolite is currently being isolated in a sufficient quantity for mass spectral and nuclear magnetic resonance determinations.

Significance to Biomedical Research and the Program of the Institute: It is hoped that the results of this project will provide information that will lead to the development of an analog of vitamin A that will display biological activity in the reversal of preneoplasia in epithelial tissues and yet will show little or no toxicity in the whole animal.

Proposed Course of the Project: Future work on this project will concentrate on the metabolism of retinoic acid with special emphasis on determining the structure and biological activity of the various metabolites formed. This, together with studies on the metabolism of various synthetic analogs, will help to determine the form of the retinoid which is responsible for

the biological activity of the retinoid molecule. Once this biologically active compound is known further work can be done to determine the mechanism whereby retinoids cause reversal of keratinization and how this process is related to the effect of retinoids on epithelial preneoplasia.

Publications

Frolik, C.A, Tavela, T.E., and Sporn, M.B.: Separation of the natural retinoids by high-pressure liquid chromatography. J. Lipid Res. 19: 32-37, 1978.

Frolik, C.A., Tavela, T.E., Peck, G.L., and Sporn, M.B.: High-Pressure liquid chromatographic determination of 13-cis-retinoic acid and all-trans-retinoic acid in human plasma. Anal. Bioch., in Press.

Newton, D.L, Frolik, C.A., Roberts, A.B., Smith, J.M., Sporn, M.B., Nürrenbach, A., and Paust, J.: Biological activity and metabolism of the retinoid, axerophthene (vitamin A hydrocarbon). Cancer Res., in Press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04585-03-LC

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Application of automated image analysis to the study of preneoplastic changes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Sherman F. Stinson Staff Fellow LC NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Lung Cancer Branch, Carcinogenesis Research Program

SECTION
Lung Cancer Pathogenesis Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 0.7	PROFESSIONAL: 0.3	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The feasibility of using an objective automated image analysis system for the study of preneoplastic morphological changes is being examined. Areas under investigation include: 1) optimization of techniques for fixation, processing, and staining of tissues and cells for histological analysis; 2) quantitation of hyperplastic responses in a variety of epithelial types; 3) quantitation of nuclear-cytoplasmic ratios in histological and cytological preparations, and measurement of changes in these ratios in response to several chemical stimuli; and (4) detection of changes in nuclear size, shape and chromatin distribution, as well as cytoplasmic alterations, in cells exfoliated from tissues exposed to various chemical stimuli.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04585-03-LC

Project Description

Objectives: This is a new project. This study is designed to evaluate the potential of an automated image analyzer for quantitating minute changes in cellular and subcellar size, shape, number and density which may have application to the detection and study of preneoplasia.

Methods Employed: A variety of epithelial tissues (including urinary bladder, trachea, and breast), as well as exfoliated cells from the lung and cultured urinary bladders, have been processed for histological use and examined on a Bausch and Lomb "Omnicon" image analyzer. Several different fixatives and stains, and 2 embedding media have been evaluated for their ability to preserve and intensify various specific morphological parameters.

Major Findings: It has been determined that the Omnicon can accurately, precisely and efficiently estimate the degree of cellular hyperplasia in an epithelium by quantitating the number of nuclei, the cross-sectional area of nuclei, or the total epithelial cross-sectional area, depending on the properties of the tissue being examined. Measurement of nuclear-cytoplasmic ratios in the urinary bladder has also been successful, and visually undistinguishable changes in the ratios caused by chemically altering the cellular environment have been detected.

Significance to Biomedical Research and the Program of the Institute: These studies will contribute to an objective, quantitated definition of preneoplasia.

Proposed Course of Project: The technology of sample preparation will continue to be refined. Further investigations into quantifiable parameters indicative of preneoplasia will be conducted. Studies on epithelial responses to chemical stimuli will also be expanded in an effort to detect early neoplastic changes.

Publications

Stinson, S.F., Sporn, M.B.: Use of an automated image analyser to quantitate cellular hyperplasia in urinary bladder epithelium. J. Microscopy 109: 329-335, 1977.

Stinson, S.F., Lilga, J.C., Reese, D.H., Friedman, D.R., and Sporn, M.B.: Quantitation with an automated image analyzer of nuclear-cytplasmic changes induced by hydrocortisone in bladder epithelium. Cancer Res. 37: 1428-1431, 1977.

Stinson, S.F., Lilga, J.C., Sporn, M.B.: Automated image analysis of nuclear and cytoplasmic changes in exfoliated cells from tracheas treated with carcinogen. Proc. Electron Micros. Soc. Am. 35: 220-221, 1977

Stinson, S. F., Sporn, M.B.: Use of automated image analysis to detect precancerous changes in the bladder epithelium. J. Appl. Photogr. Eng., in Press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-05002-01-LC

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

The effect of retinoids on carcinogenesis of the esophagus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Sherman F. Stinson Staff Fellow LC NCI

COOPERATING UNITS (if any)

none

LAB/BRANCH
Lung Cancer Branch, Carcingenesis Research Program

SECTION
Lung Cancer Pathogenesis Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to determine the effects of retinoids on esophageal carcingenesis. This goal is being pursued in 2 phases: 1) Investigations to develop and define a reliable model for esophageal carcingenisis, 2) tests to study the ability of various retinoids to modulate carcinogenesis.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: The objectives of this project are to 1) develop a reliable experimental model for the study of carcinogenesis in the esophagus, and 2) to determine the ability of retiniods to inhibit, or otherwise modify, esophageal carcinogenesis.

Methods Employed: Systemically applied methyl-alkyl nitrosamines induce neoplasms in the esophagus specifically. Several of these nitrosamines have been tested in rats to determine dose response data and the histopathology of the induced neoplasms. Serial sacrifice studies have been conducted to define the pathogenesis of the lesions.

Preliminary assays have been conducted with 3 retinoids to investigate their effects on the system.

Major Findings: Sub-cutaneous injection of methyl-benzyl nitrosamine (MBZN) induces papillary and mural neoplasms of the esophagus and pharynx. Dose-response data has been compiled; ten - fifteen weekly injections of 2.5 MBZN/kg body weight produces a high incidence of neoplasms and low mortality by 30 - 40 weeks. Evaluations of the studies investigating the pathogenesis of the neoplasms are, as yet, incomplete.

No protective effect against carcinogenesis of retinoids has been demonstrated in this system. Aromatic retinoid analogues, however, appear to enhance carcinogenesis in the esophagus.

Significance to Biomedical Research and the Program of the Institute: Esophageal cancer in humans carries a particularly poor prognosis; 5 year survival rates below 10 % are common. Development of an experimental model for the study of this disease, and investigation of agents for the inhibition are important steps in the prevention of this form of cancer.

Proposed Course of Project: Different carcinogens and species of animals will be studied to find a model whose histological and biological features more closely resemble human esophageal cancer. Further studies with alternative retinoids are proposed.

Publications

Stinson, S.F., Squire, R.A., and Sporn, M.B.: Pathology of esophageal neoplasms and associated proliferative lesions induced in rats by n-methyl-n-benzyl nitrosamine. J. Natl. Cancer Inst., in Press.

SUMMARY REPORT

BIOLOGY OPERATIONAL UNIT

October 1, 1977 through September 30, 1978

The Biology Operational Unit is responsible for developing experimental models for studying the etiology and pathogenesis of the major forms of human cancer. An integral part of these efforts and a further objective of the Unit is the use of these models for the development of methods to prevent or modify the induction and progression of these tumors. Research areas under investigation include studies on the pathogenesis of cancers of the pancreas, prostate, mammary gland, liver, colon and lung, as well as studies on perinatal carcinogenesis. Investigations are conducted at all levels of biological organization from long-term animal studies through cell and organ culture to the molecular level.

The forms of cancer chosen to be investigated and the basic fundamental concepts of carcinogenesis to be explored are selected with extensive scientific advice from outside expert consultants and from NCI staff as well as with consideration of needs in the field. The development of the program is based on an evaluation of current activities and their impact on program goals, and on new or specific ideas offered by the scientific community, particularly as they result from advances made in new research concepts and methods. The extent of implementation of the program is determined by available human and fiscal resources.

Close contact in these endeavors has been maintained with the organ site program developed in the Division of Cancer Research Resources and Centers. Significant accomplishments of the entire program in this period are described below.

Colon Carcinogenesis

The Colon Cancer Program of the Biology Operational Unit seeks to identify the etiologic factors responsible for colon cancer in humans, to develop animal and in vitro model systems for study of colon carcinogenesis, and to investigate in these systems carcinogenic processes leading to animal and human colon cancer. It combines laboratory studies with epidemiological observations in order to determine etiological elements inducing colon carcinogenesis and those factors which are involved in its control.

Epidemiological studies of populations at differing risk for colon cancer have the potential for detection of factors important in the etiology of this disease. Such studies have suggested a relationship between diet of various human populations and the incidence of colon cancer. At the Medical College of Georgia (N01-CP-43282) these studies are being extended to determine the relationship of diet (animal protein, saturated fats, foods treated with preservatives, carbohydrates, high fiber content) to

the occurrence of colorectal cancer in two southern populations. This case-control study of hospital patients in Atlanta investigates the dietary habits of both southern blacks and southern whites. These population groups have a lower than average incidence of cancer of the colon, colon cancer being less common in the South than in the North and less common in southern blacks than in southern whites. All the data has been collected and the study is now in the final data analysis phase.

Since the human microbial flora and particularly its metabolic capacity may play an important role in colon carcinogenesis, studies have been devoted specifically to identifying the intestinal microflora in populations at differing risk. These investigations attempt to determine if certain microbiological or chemical (see below) markers for risks exist, as well as if specific microbial species may play a unique role in the carcinogenic process. At the Virginia Polytechnic Institute and State University (N01-CP-33334) over one hundred diagnostic properties determined in this project have been coded and computerized for over 7000 bacterial strains facilitating the identification of the relative numbers and strains present in human populations whose risk for colon cancer differ, as well as those present in given populations under specific experimental conditions; for example, under defined dietary regimens. At least 350 bacterial species have been encountered in these studies among more than 11,000 isolates. A change in diet itself does not necessarily alter the microbial genera since data indicate that diet is not instrumental in controlling the composition of the flora. Preliminary analyses suggest, however, that there are some statistical differences in flora composition that correlate with risk for colon cancer; and that one group of bacteria has a negative correlation with risk. Overall, however, all types seem to be present in some degree irrespective of risk classification. High meat consumption increases the proportion of anaerobes. This is not mediated through high cholesterol intake since evidence indicates no increase in cholesterol or its metabolites is found in the excreted solids. Data have established that none of the gastrointestinal tract is sterile, that the major bacterial growth occurs in the cecum and ascending colon, and that the human fecal flora is representative of the colonic flora.

Epidemiologic evidence shows a high correlation of colon cancer incidence with diet, especially consumption of meat and animal fat. One effect of a high meat-high fat diet is to raise the concentration of neutral steroids (such as cholesterol) in the colon; and it has been suggested that anaerobic bacteria in the colon might produce carcinogenic intermediates from these compounds. In a companion contract at Virginia Polytechnic Institute and State University (N01-CP-55685) efforts have been focussed on this and other possible biochemical markers of colon cancer risk.

Concentrations of neutral steroids excreted by 10 populations at different risk for colon cancer have been determined. Concentrations of animal steroids were higher in the feces of populations at high risk for colon cancer than in those of populations at low risk for colon cancer. No such differences were found in concentrations of fecal plant steroids.

Bacteria in the colon of most people convert cholesterol into coprostanol and coprostanone, but the extent of conversion varies appreciably from person to person. It has been found that among normal North Americans two distinct conversion patterns exist: a high conversion pattern (>50% conversion of available cholesterol into coprostanol and coprostanone) and a low conversion pattern (<50% conversion). This is the first known report of the existence of the low conversion pattern for a presumptively normal population.

The conversion pattern for low-risk rural black South Africans was found to differ from all other populations studied in that nearly all (98%) individuals observed were high converters of cholesterol.

Appreciable concentrations of long chain fatty acids are found in human feces. Since these compounds can aid in the solubilization of neutral steroids, the relationship between long chain fatty acid concentrations and cholesterol reduction in the feces of people from four populations at different risk was determined. No correlation was found between fatty acid concentrations and cholesterol reduction, but the concentrations of oleic acid (C18:1) were higher in the feces of two high-risk populations than in the feces of the two low-risk populations.

The fate of neutral steroids in the human gastrointestinal tract has been investigated by determining their concentration in intestinal contents at 7 to 8 levels of the human gastrointestinal tract. Cholesterol conversion occurs mainly in the ascending colon, an assumption for some years but not previously proven in humans. In addition, the assumption that concentrations of neutral steroids in feces are similar to concentrations throughout the colon was shown to be correct.

Organisms which reduce cholesterol to coprostanol use cholesterol as a terminal electron acceptor. The nature of the terminal electron acceptor affects the energy that an organism can obtain from its substrates. In general, bacteria will utilize the electron acceptor which gives the greatest energy advantage. Nitrate and fumarate are electron acceptors which are used by many facultative and some obligate anaerobes to gain energy. But their levels in the colon are probably very low. Sulfate is thought to be more common especially since colon mucin contains considerable amounts of sulfate and mucin appears to be broken down by colonic bacteria. Contractor has made the first discovery of an anaerobic sulfate-reducing organism in human feces. The organism contains a c-type cytochrome, and possibly a b-type, as well as the pigment desulfoviridin.

A major recent finding in this project (Virginia Polytechnic Institute and State University; N01-CP-55685) has been the determination that mutagens, extracted from feces with ether in the neutral steroid fraction, are more prevalent in populations at high risk for colon cancer than in populations at low risk. Ten percent of a group of North Americans and 19% of an urban, white South African group (both high-risk populations) excreted significant amounts of mutagenic substances, whereas in two low

risk populations, rural and urban black South Africans, 0% and only 2% excreted significant amounts of mutagens. Preliminary data on small numbers of people over a limited period of time appear to indicate that mutagen excretion (or non-excretion) may be a characteristic of the individual. Other studies on fecal mutagens investigating their production and inactivation have found that the mutagenic capacity of fecal extracts can be altered by colon bacteria, mammalian enzymes, and by fiber and mucin.

At the Memorial Hospital for Cancer and Allied Diseases (N01-CP-43366) emphasis continues to be placed upon indices and markers defining stages of preneoplasia in cells of human population groups at high risk for colon cancer. New population registries have been developed of individuals who are at increased risk for colorectal cancer. Diseases recorded include familial polyposis, familial colon cancer, primary colon cancer, and multiple cancers (with colonic, breast and endometrial neoplasms in highest frequencies). Analyses of the data in these registries using the Kolmogorov-Smirnof 2-sample test show the interesting relation that several of these disease groups (multiple cancers, familial colon cancer, familial polyposis) have different ages of onset of colorectal cancer among themselves and earlier than in the general populations of the United States and Japan.

A number of interesting results have been found in other studies directed at identifying phenotypic abnormalities that develop in human cells as they undergo neoplastic transformation. Abnormal proliferation of histologically normal colorectal mucosa has been found in tissue from patients with colorectal cancer. Autoradiographic analysis of ^{3}H-TdR incorporation showed a highly significant shift of the proliferative cell compartment to the middle and upper thirds of the crypts of Lieberkuhn rather than in the lower third as observed in controls. The detection of this patchy alteration of epithelial cell renewal, frequently accompanying primary colorectal cancer, may provide a useful discriminatory parameter in studying relative risk of this population group.

In the cytoplasm of well spread cultured normal fibroblasts, actin is organized into a network of cables that run the length of the cell just inside the adherent cell membrane. A disorganization of actin occurs in fibroblasts that have become tumorigenic as a result of oncogenic transformation. A disruption in actin organization was found in cultured skin fibroblasts obtained by biopsy from individuals with familial polyposis. Findings indicate that immunofluorescent localization of actin within cultured skin fibroblasts may be useful in the early identification of affected individuals.

Studies directed at possible markers for latent inherited polyposis have found that skin fibroblasts from polyposis subjects in cell culture are 100-1000 fold more susceptible to transformation (focus formation) with Kirsten murine sarcoma virus than normal skin cells. In similar

studies searching for biochemical markers, studies of chromosomal proteins of fibroblasts by electrophoresis in SDS gels have shown differences between chromosomal proteins from desmoid tumor and those from fibroblasts derived from flat skin.

Other studies in cultured human colon carcinoma cells have shown that 10 to 15% of pulse-labelled nuclear RNA is protected from exogeneous nuclease, that much RNA exists in a ribonucleoprotein structure which is very rich in guanosine and cytidine, and represents a specific subset of all sequences in heterogeneous nuclear RNA.

The American Health Foundation (N01-CP-33208) continues to develop animal models for the study of colon carcinogenesis with emphasis upon total dietary fat, hidden fat as present in meat, dietary fiber, and the role of the intestinal bacterial flora and of acid and neutral steroids. Important studies of bile acid promotion of colon carcinogenesis have shown that the primary bile acids, cholic acid and chenodeoxycholic acid, act as colon tumor promoters in conventional F344 rats but not in germ-free animals. This may be due to production of deoxycholic acid and lithocholic acid by the bacterial 7α-dehydroxylation of cholic acid and chenodeoxycholic acid in the colon of conventional rats extending and confirming this contractor's previous findings that the secondary bile acids, deoxycholic acid and lithocholic acid, promote colon cancer induction in germ-free rats. Neutral sterols, such as cholesterol, cholesterol epoxide and cholestan -3β-5α, 6β-triol do not act as colon tumor promoters in germ-free or in conventional F344 rats.

Since these experiments tested the promoting activity of intrarectally-administered bile acids in the presence of the normal luminal bile flow and the luminal content flow generally, additional experiments are investigating the promoting ability of the bile flow (luminal flow) itself on the one hand and of certain bile acids by themselves in the absence of luminal flow on the other. Rats bearing colostomies are employed in both types of experiments. Results to date indicate that luminal flow is necessary for carcinogenesis induced by interrectal application of the direct-acting carcinogen methylnitrosourea (MNU). An experiment testing for the promoting ability of deoxycholic acid on methylazoxymethanol induced carcinogenesis in the absence of luminal flow is still in progress.

The effect of dietary fiber on colon carcinogenesis has been determined in a series of experiments in which wheat bran, pectin and alfalfa were the sources of fiber and azoxymethane (AOM, which requires metabolic activation) and MNU were the carcinogens. The effect of the food additives carrageenan and tannic acid on colon carcinogenesis induced by these agents was also investigated. Results indicate that pectin, bran and tannic acid inhibit AOM-induced tumorigenesis but that none of the dietary fibers (or additives) inhibit tumorigenesis induced by MNU. The carrageenan sample which was used, in fact, had a marked promoting effect on colorectal carcinogenesis evoked by either AOM or MNU, and alfalfa appears to promote to some extent MNU tumorigenesis.

Recent evidence has emphasized the importance of cooking procedures in the entire perspective of dietary practices and gastrointestinal tract carcinogenesis. It has beeh shown that mutagenic substances can be isolated from pyrolysates of the amino acids trytophan and phenylalanine. Efforts in this contract with a similar objective have resulted in the development of high pressure liquid chromatographic methods for the analysis of benzo(a)pyrene in charcoal broiled meat and in rat and human feces. Application of the procedures to rats has already been made and work in humans is in progress.

Two new programs in the colon carcinogenesis area have been initiated recently emphasizing the target organ itself, the colonic mucosa. Two contracts are devoted to the studies of colon carcinogenesis in organ culture in which development of new methods for organ culture of the colonic mucosa will be pursued along with studies of the mechanisms and pathogenesis of colon carcinogenesis and anticarcinogenesis. At the American Health Foundation (N01-CP-75952) techniques have already been developed for maintaining mouse and rat descending colon in culture for up to 28 days with a viable and functional mucosal epithelium, and with a relatively normal pattern of replication and turnover. Preliminary studies show that the colon carcinogens 1,2-dimethylhydrazine, azoxymethane and methylazoxymethanol acetate inhibit mucosal replicative DNA synthesis and that N-methyl-N^1-nitro-N-nitrosoguanidine induces DNA repair in explants in which replicative synthesis is suppressed by hydroxyurea.

In a companion contract at the IIT Research Institute (N01-CP-75953) novel culture techniques for maintenance of adult rat colonic mucosa are employed, including a human fibrin foam matrix as well as a grid technique using a membrane filter on a stainless steel platform. Viability has been maintained for extended periods of time as determined by measurements of glycoprotein synthesis and incorporation of tritiated thymidine and leucine into DNA and protein.

Studies in the second new program this year are focussed on the metabolic capacity of the intestinal mucosa. At the Sloan-Kettering Institute for Cancer Research (N01-CP-75950) studies are focussed on regulatory aspects of cellular metabolism in cell growth and differentiation. Emphasis is placed upon early changes in biochemical parameters with special attention to multivalent allosteric enzyme systems and their subcellular activity distribution in normal and carcinogen-induced tumor cells. It has been found that 5-phosphoribosyl-1-pyrophosphate synthetase is totally membrane-associated in rat cells in contrast to previous reports that in human erythrocytes this important enzyme in the first steps of nucleotide and deoxynucleotide biosynthesis is cytosolic. Other efforts explore the role of polyamines in cell differentiation and transformation.

At the American Health Foundation (N01-CP-75948) the metabolic activity of the luminal contents, colonic mucosa and other tissues are studied in efforts to relate the organospecificity of colon carcinogens to metabolic activation pathways present exclusively or in greater levels in the colonic mucosa as compared to other organs. Initial results indicate that

three colon carcinogens are metabolized under anaerobic conditions by cecal contents of conventional rats but not by such preparations from germ-free animals. In the case of 1,2-dimethylhydrazine, monomethylhydrazine has been tentatively identified as a metabolite. Studies of metabolism by rat liver preparations in vitro show that the colon specific carcinogen azoxymethane is not only hydroxylated to methylazoxymethanol but that a second product is also formed. Previous work on the inhibition and mechanism of inhibition of 1,2-dimethylhydrazine metabolism by disulfiram is now being extended in studies of metabolism of 3,2^{1}-dimethyl-4-aminobiphenyl by liver S-9 fractions. Binding of metabolite(s) occurs to S-9 constituents which is decreased 50% when S-9 fractions from disulfiram-pretreated rats are used.

At the Sloan-Kettering Institute for Cancer Research (N01-CP-75951) the influence will be studied of diet, enzyme inducers, and genetic constitution on metabolism of chemical carcinogens by the colonic mucosa of man and rodents. Early results indicate a low level of dimethylnitrosamine demethylase activity in uninduced colonic mucosa of certain mice and an inducible benzo(a)pyrene hydroxylase in mice and rats.

Studies at the New York Medical College (N01-CP-75949) will emphasize the capacity of the colonic mucosa and representative anaerobes of the intestinal microflora to biotransform compounds to mutagenic and DNA-modifying substances.

At the Frederick Cancer Research Center efforts are directed at the identification of exogenous and endogenous substances which may be metabolized to carcinogens, mutagens or cocarcinogens in the gastro-intestinal tract. The biotransformation by intestinal tract microfloral and mucosal enzyme systems and by liver microsomal enzymes of neutral sterols, bile acids, azo dyes, aromatic amines and polynuclear aromatic hydrocarbons is studied. The interesting finding has been made that lithocholic acid (LA), a major human fecal bile acid, and a tumor promoter of colon carcinogenesis in rats, enhances the mutagenicity in the Ames test of 2-aminoanthracene and benzo(a)pyrene when a phenobarbital-induced rat liver S-9 fractions is used. Lithocholic acid apparently transforms hamster embryo cells also and comparison of its metabolism with that of its sulfate ester and taurine conjugate indicates unique metabolism of each of these bile acids by the human intestinal microflora. The potential importance of the human microflora is also shown by its capacity to produce mutagenic metabolites from azo dyes which are not mutagenic in the Ames assay either in the presence or absence of a metabolic activation system.

At the University of Nebraska, Eppley Institute for Research on Cancer, the interesting finding has been made that N-nitrosobis (2-oxopropyl) amine (BOP) injected subcutaneously into MRC rats induces colorectal tumors which closely resemble the equivalent lesion in man. This nitrosamine is a known potent pancreatic carcinogen in hamsters; in rats, however, it does not induce pancreatic tumors. In the MRC rat, colorectal

cancer is induced in 67% of all males and in 33% of all females surviving beyond 43 and 68 weeks, respectively. The potential value of this MRC rat-BOP system as a model for colorectal carcinogenesis is undergoing further evaluation.

Prostate Carcinogenesis

The development of model systems for studies in prostate carcinogenesis continues to occupy a central position in activities of the Biology Operational Unit. Investigations to this end for nearly forty years have met with minimal success. As part of these efforts, a major emphasis is placed upon the area of normal prostatic cell culture and upon androgen metabolism in animal and human systems.

At the Pasadena Foundation for Medical Research (N01-CP-65850) the focus is upon development of procedures for establishment and characterization of primary cultures of normal, human prostatic epithelial cells. Normal human neonatal prostatic epithelial cells have been successfully subcultured for the first time and have undergone at least 45 population doublings. These cells have normal chromosomes by fluorescence banding and normal ultrastructure by electron microscopy. Nutritional studies have determined adequate media for isolation and propagation of normal prostate cells. Epidermal growth factor and fibroblast growth factor increase both the growth rate and lifespan of these cells. With at least one cell line, these factors act synergistically, an interaction not seen in prostate-derived fibroblasts. In this line neither insulin nor vitamin A affect the clonal growth rate or plating efficiency. These normal prostate cells have testosterone 5-alpha reductase activity, are stimulated by testosterone to incorporate tritiated thymidine into DNA, and specifically bind labelled testosterone metabolites in the nucleus. In this work a novel technique has been developed for subculturing epithelial cells. The method employs an elevated concentration of potassium ions and high osmolality rather than enzymes. The method has been used to subculture normal epithelial cells from the prostate for the first time.

In vitro cultivation of normal, epithelial human prostatic cells is pursued also in a second project at the University of Colorado (N01-CP-65849). A method has been developed for separation of prostatic acini from stroma using collagenase and this procedure has been used to establish vigorously growing cultures of prostatic epithelium. Optimum nutritional levels have been established for growth of normal human prostatic epithelium including: type of medium, type and concentration of serum and concentrations of glutamine, insulin and 5α-dihydrotestosterone. Cells are characterized at the ultra-structural level, cytochemically for prostatic acid phosphatase and by indirect immunofluorescence using antiserum against prostatic acid phosphatase. Growth of cultures in these studies has been quantitated by a new method using a color scanning densitometer.

At the W. Alton Jones Cell Science Center (N01-CP-65762) efforts are devoted to cultivation and characterization of normal rat prostatic epithelial cells. Procedures have been developed for preparing epithelial cells which employ collagenase dissociation and differential attachment. Epithelial cells obtained incorporate thymidine as demonstrated autoradiographically, possess numerous secretory vesicles and tonofilaments and are joined by tight junctions and desmosomes. Optimum conditions for nutrition of these cells are explored and histochemical procedures for enzyme localization and other markers are being worked out.

At the Southwest Foundation for Research and Education (N01-CP-33379) emphasis is placed upon prostate androgen metabolism and prostate androgen receptors as a function of age in normal, lesion-free senile prostate and in neoplastic specimens. Studies employ rat, dog and human tissue. Such studies may contribute to knowledge of regulation of prostate epithelial cell function. The discovery was made in this project of spontaneous adenocarcinomas of the ventral prostate of the AXC rat which reach a frequency of 70% in animals greater than 30 months of age. Since a satisfactory animal model for prostatic carcinogenesis studies has yet to be developed, this finding has great potential importance and is being pursued in ongoing investigations.

The ventral prostate of the aging AXC rat which develops spontaneous adenocarcinoma is characterized by decreased cytoplasmic and nuclear androgen receptor content, diminished capacity to synthesize 5α-dihydrotestosterone, increased capacity to synthesize Δ^4-androstenedione and diminished dependence on androgen for maintenance of cell number.

Testosterone metabolism by the prostate of the aging dog, however, shows an aging-related increase in 5α-dihydrotestosterone production, a decrease in 4-androstenedione production, and the absence of an aging-associated decrease in receptor content per prostate cell, changes which are in complete opposition to those which characterize the ventral prostate of the aging rat.

Studies of testosterone metabolism by human benign hyperplastic prostate (BPH) demonstrate that this tissue has a greater reductive testosterone metabolic capacity than either senescent canine or rodent prostate. Human BPH was the only tissue in which in vitro exposure to estradiol causes a shift to oxidative testosterone metabolism, primarily due to decreased 5α-dihydrotestosterone production. In human prostate specimens, procedures have been developed to reliably determine both occupied and unoccupied androgen receptors, both cytoplasmic and nuclear, with small amounts of tissue.

Medrogestone has been found to be a highly effective antigonadal agent for the production of precocious prostatic senility. The relation of precocious prostatic senility to early induction of adenocarcinoma of the rat ventral prostate will be determined.

Lung Carcinogenesis

The Lung Cancer Program of the Biology Operational Unit is devoted to studies of the pathogenesis of lung cancer and its prevention. The ultimate goal of the Program is the prevention of development of lung cancer in man. A variety of experimental techniques are used in many investigations, ranging from long-term animal studies to short-term morphological and biochemical experiments. All of these efforts are coordinated to establish a correlation between animal models and human lung cancer, and to develop a targeted approach to the inhibition or prevention of this most prevalent form of fatal cancer in man. The need for such a program to lessen the incidence of this widespread, lethal disease scarcely requires further comment. During the past year major investigations in the areas of in vitro carcinogenesis in respiratory epithelium and in several aspects of retinoids research have further developed. Significant accomplishments of the entire program in this period are described below.

At the Oak Ridge National Laboratory (DOE-NCI Interagency Agreement Y01-CP-50200) the pathogenesis of lung cancer is being investigated in a number of long-term animal studies that are of direct relevance to the human disease. Studies of nitrosomethylurea (NMU)-induced tracheal tumor incidence employing a specially designed tracheal catheter for carcinogen delivery have established a number of protocols in which frequency of NMU-administration and NMU concentration were varied such that tumor incidences from 20% to 90% can now be achieved with tumor induction times of 10 to 50 weeks. Histologically, adenocarcinomas were found to outnumber other tumor types at low NMU concentrations.

This tracheal washing procedure for respiratory tract carcinogenesis was employed to study the effects of two retinoids (13-*cis*-retinoic acid and the ethyl amide of the trimethylmethoxyphenyl analog of retinoic acid). To date significant inhibition of tumor development is not seen at the three levels of NMU exposure chosen giving from 55% to 80% carcinoma incidence in the control groups.

The tracheal transplant system previously developed at ORNL to investigate the role of cofactors in respiratory tract carcinogenesis continues to be employed in important studies: a) nickel subsulfide appears to be a weak carcinogen for epithelium in this system, but is highly sarcomagenic when given in large amounts; b) chrysotile asbestos appears to be a weaker carcinogen; c) the potent tumor promoter 12-O-tetradecanoyl phorbol-13-acetate (TPA) is under study for its promoting effect on dimethylbenzanthracene (DMBA)-induced tumorigenesis; and d) lesion development in DMBA-exposed tracheas is under critical evaluation as a function of time with tumor incidence under the same experimental conditions and endpoint also.

Immunological studies show that the majority of respiratory tract carcinomas of rats and mice induced by polycyclic hydrocarbons are antigenic, that many of these carcinomas have cross-reacting antigens which are neither embryonal nor viral in origin and that antigenicity increases markedly with extended culture.

A number of interesting studies are pursued employing tracheal transplants, tracheal organ culture, cell culture of tracheal cells and combinations of these technics: Tracheal cells exposed for a relatively short time "in transplant" to a higher dose of DMBA undergo transformation earlier in culture than those exposed to a lower DMBA dose; the shorter the time to transformation in culture, the shorter the tumor induction time in animals; the higher the "in transplant" DMBA dose, the greater the degree of primary culture and cell line establishment.

A new tracheal repopulation technique has been developed to test the differentiative and oncogenic capacity of epithelial cells. Tracheas with intact, partially or totally denuded epithelium which are repopulated with normal cell suspensions exhibit a normal mucociliary epithelium. Preneoplastic and neoplastic cell lines invaded the surrounding tissues. The presence of normal epithelial cells forms an effective, but not absolute, barrier to neoplastic cells.

Studies in an organ culture-cell culture system demonstrate that promotion of MNNG carcinogenesis in tracheal organ cultures by TPA can be achieved. Transformation frequency is increased and "latency" in culture for oncogenic transformation decreased. It was also shown that TPA alone permanently alters the growth-regulating mechanisms of epithelial cells: Cell lines derived from TPA-exposed tracheal organ cultures are similar to those obtained from carcinogen-exposed cultures, but they are not tumorigenic.

At the Harvard School of Public Health (N01-CP-33273), the synergism between radiation and chemical carcinogens in causing lung cancer is being studied. Such synergism has been implicated in the high incidence of occupational lung cancer in uranium, hematite and flurospar miners; and possibly also in the smoking population. Significant investigations on the distribution of carcinogens in the respiratory tract combined with long-term carcinogenesis studies have shown that the segmental distribution of lung cancer induced in hamsters by the intratracheal instillation of either polonium-210 or benzo(a)pyrene (BP) adsorbed onto hematite particles, as well as BP administered in gelatin-saline suspension, can be related to the transport and localization of each agent in the respiratory tract. It has been found that mean alpha radiation doses to the lung from polonium-210 of only 15 to 75 rads produces lung cancers in hamsters. A significant synergistic interaction between low doses of Po-210 and BP appeared to occur when BP exposure followed Po-210 exposure by 4 months. Most of this effect could be ascribed, however, to a potentiating effect of subsequent saline instillations on Po-210 carcinogenesis. Preliminary data suggest that the saline instillations act to stimulate cell proliferation in the lung. These results emphasize the fact that seemingly innocuous stimuli may significantly potentiate lung carcinogenesis, an observation which may have important implications in terms of the interactions between alpha radiation and cigarette smoke in human populations.

At the New York University Medical Center (N01-CP-33260), the major emphasis is on the identification of hazardous materials which may pose a serious threat to man as respiratory carcinogens. Great effort has

been spent in the design and establishment of an inhalation chamber facility which can accomodate over 1000 animals. Systems for the generation of aerosols, vapors, and industrial dusts have been developed for testing the carcinogenicity of many substances by the inhalation route. Among the important substances which have been found to be carcinogenic or cocarcinogenic for the respiratory tract are calcium chromate dusts, sulfur and nitrogen dioxide, dusts formed from polyurethane foam (workers in the building trades industry may be exposed to this type of dust), and bischloromethyl ether, a common industrial chemical. Results in long-term animal studies show that croton oil inhalation following methylcholanthrene or benzo(a)pyrene intubation in hamsters results in an increase in respiratory tract cancer incidence compared to that in animals receiving carcinogen alone. In lifetime exposures to the alkylating agent dimethylcarbamoyl chloride at 1 ppm, 94 of 98 rats incurred squamous cell carcinomas of the nasal cavity with all animals dead by 52 weeks. This tumor is also appearing in rats now being chronically exposed at 0.3 ppm but with longer induction times. Hamsters, currently undergoing exposure at 1 ppm, are also developing squamous cell carcinoma of the nasal cavity. This incidence, however, is much lower than seen with rats at this level (25/60 to date) and appears much later. Regulating agencies, industry in the United States and overseas, and the scientific community have been informed of these results and will be made aware of future developments as these studies progress.

Similarly, epichlorohydrin, another widely used industrial intermediate and alkylating agent, is currently under test in rats and is showing carcinogenic effects in the respiratory tract. These preliminary results have also been made available to the federal regulating agencies, industry and the scientific community.

Since many projects in the lung cancer program involve long-term animal studies in defined models of lung carcinogenesis and modulation of epithelial carcinogenesis in other systems, a contract provides for the supply of animals treated with respiratory and other carcinogens (ITT Research Institute [N01-CP-43289]). This contract prepares and delivers Syrian golden hamsters and rats treated under protocols leading to carefully controlled conditions for the initiation of preneoplastic lesions and tumors of the respiratory tract and bladder. The various treatment regimens and periods of treatment are used to produce a variety of preneoplastic lesions. As part of these efforts, a major effort has been directed toward the further development and evaluation of a new method for inducing neoplastic lesions of the respiratory tract. The technique permits the induction of a cancer in a circumscribed region of the hamster trachea which is similar to bronchogenic carcinoma in humans. A catheter was fabricated which delivers the carcinogen (N-methyl-N-nitrosourea, MNU) to approximately a 5mm length of the trachea 10-15mm distal to the vocal cords. The treatment of 7-week-old hamsters 1X/week for 15 consecutive weeks with either 0.5 or 0.25% MNU induced a 68% and 33% incidence of tracheal cancers, respectively, within 6 months. This regimen appears ideal since it permits the treatment of a large number of animals without toxic effects.

At the IIT Research Institute (N01-CP-23292) major long-term animal studies continue on the modulation of epithelial carcinogenesis by retinoids. Retinyl acetate and retinyl methyl ether have been shown to suppress the appearance of mammary adenocarcinomas induced in Sprague-Dawley female rats by either 1-methyl-1-nitrosourea or 7,12 dimethylbenz (a)anthracene. In studies in which either a low or high incidence of cancers were induced, these retinoids significantly prolonged the latency of cancer appearance and reduced the average number of cancers per rat. Furthermore, it was demonstrated that continual dietary supplementation with retinyl acetate was necessary to maintain retinoid chemopreventive activity and that retinyl acetate appeared to exert its effect by inhibiting the progression of early neoplastic lesions. Other retinoids were found to be ineffective in either of these systems in inhibiting mammary carcinogenesis.

Animal models have also been developed for use in the study of transitional cell and epidermoid carcinoma of the urinary bladder in rats and mice, respectively. A dose dependent induction of urinary bladder cancer with N-butyl-N-(4-hydroxybutyl)-nitrosamine (OH-BBN) using a quantitative dosing schedule has been achieved. In Fischer rats feeding 13-cis-retinoic acid after completion of carcinogen treatment diminished the number and severity of cancers and other proliferative lesions of the urinary bladder. Similarly, feeding of 13-cis-retinoic acid to C57BL/6 male mice after completion of OH-BBN treatment prevented the development of both cancers and benign tumors.

Models have also been developed for inducing esophageal and respiratory tract (tracheal) cancers in Fischer 344 rats and Syrian golden hamsters respectively. To date, of the retinoids tested no significant chemopreventive activity against the induction of esophageal cancers has been found with evaluation in the tracheal system still underway.

Since a significantly large number of deaths from lung cancer in man are caused by oat cell carcinoma (which is a particularly invasive and metastatic type of tumor), efforts at the Dartmouth College Medical School (N01-CP-65776) emphasize the development of an animal model for this particular respiratory tract cancer. Efforts take two directions, either through xenogenic transplantation of human oat cell carcinoma cells from established cell lines or through primary induction of an analogous type of animal tumor. Results from the first approach have evaluated growth characteristics of the cell lines in animal hosts, including the effect of the sex of the host and administration of antilymphocyte and antithymocyte serum. Efforts directed at primary induction of small cell anaplastic carcinoma in the lung have begun using subcutaneously applied diethylnitrosamine in the hamster as the experimental model.

To effectively pursue many of the above studies on lung tissue from both man and animals, improved cytological methods, such as autoradiography, are being conducted at the VA Hospital in Tampa (Y01-CP-55625). The technique of light microscopic autoradiography of the respiratory tract has been improved, so that the actual amount of radioactivity

in cellular and subcellular compartments of respiratory epithelium can be quantitatively measured. It has been found previously that intratracheal administration of BP, as well as vitamin A deficiency, causes an increase in cellular proliferation in respiratory epithelium, as quantitated by thymidine labelling. In addition, it has been found that vitamin A deficiency causes loss of ciliated cells and an increase in the number of basal cells in respiratory epithelium. High doses of vitamin A cause an increase in ciliated cells.

The in vivo cellular and subcellular localization of ^{3}H-BP in tracheal epithelium of normal and retinoid-deficient hamsters has been studied. Total radioactivity in deficient hamsters was greater than in retinoid sufficient hamsters in the basal and suprabasal regions of squamous metaplasia. However, no significant differences were observed in specific activity of nuclear or cytoplasmic compartments between normal animals and either squamous metaplastic or nonmetaplastic areas of deficient animals.

A major study on in vivo cell kinetics in adult hamster tracheal cells has employed many separate methods for determining cell cycle parameters: fraction of ^{3}H-TdR labeled mitoses; double-labeling with ^{3}H and ^{14}C-TdR; and grain counts over interphase nuclei as a function of time following a single ^{3}H-TdR injection. Quantitative autoradiographic procedures were employed. Ciliated cells were not labeled nor observed in mitosis. Definitive values have now been established for basal and mucus cells for lengths of S, G2, M, G1, cycle time, growth fraction, labeling index, and mitotic index. The fraction of cells dividing very slowly or "resting" (GO) was also calculated for basal and mucus cells; and the lifespans of these cells estimated (basal 104 days; mucus 119 days; ciliated about 130 days). Findings in these studies suggest that ciliated cells arise by differentiation of mucus cells rather than by basal cells.

Further work extends these studies to changes observed in the tracheal epithelium following administration of the direct acting carcinogen NMU by the tracheal washing procedure. The extent of changes in the tracheal epithelial cell populations increased with increased number of NMU applications: undifferentiated "luminal" cells replaced both mucus cells, which rapidly decreased, and ciliated cells which decreased more slowly; with three or more applications of NMU giant basal, giant "luminal" and giant ciliated cells were observed, and squamous metaplasia; and after five applications increases in average size of cells of a given type, absence of normal cellular differentiation, labeling indices for the various kinds of cells 5 to 40 times greater with carcinogen than corresponding number of buffer applications, and labeling data indicating that some cells either were not dividing or had greatly lengthened cycle times. With NMU treatment the tracheal cell population consists of giant basal cells, giant ciliated, giant "luminal", squamous, suprabasal and ciliated cells which to date do not appear to be proliferating; and normal-appearing basal cells and "luminal" cells which seem to be the main proliferative compartments. Although these new cell types appear after NMU-treatment, they seem unlikely to be precursors of neoplasms if other cell kinetics methods now being employed confirm their nonproliferative

status. From a parallel tumorigenesis experiment using this once a week, 15-week treatment protocol, it is known that squamous cell carcinomas (and one adenocarcinoma) are present in tracheas of all hamsters sacrificed 7 or 8 weeks post-treatment.

Investigations are pursued in two projects devoted entirely to the study of respiratory carcinogenesis in vitro. At the State University of New York at Stony Brook (N01-CP-33361), methods have been developed for large-scale, long-term organ culture of rat tracheal tissue. As many as 300-500 cultures can be prepared at a time and under suitable conditions grown for periods of months. Cultures are uniform in appearance and in ability to respond to various stimuli by cellular repair and by mitotic activity. A number of types of media have been investigated in studies of epithelial growth and maintenance of differentiation. The role of serum in epithelialization of the free floating cultures was studied. Serum was shown to facilitate migration of epithelial cells as a continuous flattened sheet. Cells do not divide while migrating. Parallel events in epithelial wound healing were shown to occur in culture as in vivo.

A series of protocols for exposure of cultures to carcinogens were investigated. Continuous exposure to BP or periods of exposure alternating with periods of carcinogen-free medium was found to result in shortening of the life of the culture in rough proportion to the concentration of carcinogen. Cultures exposed continuously for two weeks to BP, and then removed to carcinogen-free medium, developed areas of focal hyperplasia which may be equivalent to nodular hyperplasias in livers of carcinogen-treated animals. These areas persist for at least six weeks, and are reversed or prevented from appearing by retinyl acetate, retinoic acid and three of four analogues of retinoic acid tested.

Ultrastructural, biochemical, and cell cycle kinetic studies are performed to identify markers of malignant transformation before diagnostic histologic changes appear. It has been observed that the cells of carcinogen-induced hyperplastic regions secrete plasminogen activator and have abnormal plasma membrane structure. Lanthanum staining and freeze fracture have revealed extensive tight junctions in the epithelium, as well as structural abnormalities of the plasma membranes whose characteristics are being studied. Comparisons are made with untreated cultures, normal tracheal epithelium, regenerating tracheal epithelium, tracheas treated in vivo with BP, and tracheal tumors arising in vivo.

Companion monolayer culture studies are performed of cells derived from carcinogen-treated cultures, including growth characteristics, ultrastructure and cytogenetics, and comparisons made to untreated cultures and to normal, freshly excised tissue. After carcinogen exposure, explantation is more vigorous and the cells are pleiomorphic.

In the second project, organ culture studies at the University of Vermont (N01-CP-33360) have systematically explored various chemically-defined media and the effects of serum, insulin, retinoids, and adrenal cortico-

steroids on long-term cultures of hamster tracheal epithelium. Explants maintained in "complex" chemically defined medium with serum exhibit marked epithelial hyperplasia and dedifferentiation, whereas tissues maintained in minimal basal medium with serum retain a differentiated appearance. Squamous metaplasia develops in the absence of serum or retinoids. In a retinoid-free medium non-essential amino acids, especially serine and glutamic acid, promote and strikingly enhance the development of squamous metaplasia. Nutritional requirements of both differentiated and squamous metaplastic epithelium have been explored in vitro with cultures maintained for as long as six months.

In other studies, anaplastic carcinomas (sometimes with fibrosarcomatous elements) have been regularly induced in hamsters by implants of tracheal organ cultures exposed to inorganic dusts and 3-methylcholanthrene (MCA). The cocarcinogenic effects of ferric oxide and crocidolite asbestos have been compared: with asbestos, tumors can be induced with 10- to 20-fold lower concentrations of MCA than with ferric oxide. This corresponds to less than 0.1 μg MCA per 4 mm^2 of epithelial surface. Comparative experiments with kaolinite and carbon as carriers of carcinogens are in progress.

Exposure of hamster tracheal organ cultures to crocidolite or amosite asbestos has been found to produce basal cell hyperplasia and squamous metaplasia. Incorporation of tritiated thymidine is enhanced in cultures exposed to amosite asbestos (and less so but still significantly in cultures exposed to crocidolite asbestos) compared to control cultures. Retinyl methyl ether in these cultures at 10^{-7}, 10^{-8}, and 10^{-9} M inhibited DNA synthesis and metaplastic changes in a dosage-dependent manner.

Since it is well established that many carcinogens (including those which affect the lung) must be metabolically activated before they can exert their carcinogenic effects, a major effort has been developed in this area. Such research has long-term potential to find effective means to block the conversion of inhaled or ingested carcinogens to metabolically active forms, and thus to inhibit the development of lung cancer. Three contracts study polycyclic hydrocarbon metabolism in the respiratory tract.

At the University of Minnesota (N01-CP-33364), efforts are focused on the role of antioxidants, such as butylated hydroxyanisole (BHA), in the inhibition of pulmonary neoplasia resulting from administration of polycyclic hydrocarbons. Studies on the mechanism of inhibition of carcinogen-induced pulmonary neoplasia by BHA have shown that this antioxidant alters microsomal metabolism so as to enhance carcinogen detoxification. Studies of the microsomal metabolism of BP using an improved high-resolution, high-pressure liquid chromatographic technique have resulted in several striking findings using liver microsomes from mice fed BHA: an almost total loss of formation of BP-4,5-oxide and of 9OH-BP with an increase in formation of 3OH-BP. Use of microsomes from animals which had received BHA shows a reduced metabolism of BP to metabolites binding to DNA. This reduction in binding occurs with microsomes from all eight strains of mice investigated. Moreover, with the known

proximate carcinogenic metabolite BP-7,8-dihydrodiol as substrate, BHA again causes a reduction in binding to DNA. This inhibition is presumably of the reaction converting the 7,8-dihydrodiol to the DNA binding metabolite BP-7,8-dihydrodiol-9,10-oxide and would appear to be of considerable importance. Butylatedhydroxyanisole inhibition of BP metabolite binding to lung DNA has also been shown in in vivo experiments.

A modification of the Ames Salmonella typhimurium mutagenesis procedure has been developed which has the potential for identifying compounds likely to inhibit carcinogen-induced pulmonary neoplasia. It has been found that microsomes from mice which have received BHA produce less mutagenic metabolites of BP than microsomes from corresponding controls.

At the University of Stockholm (N01-CP-33363), efforts are directed at defining the relationship between the microsomal enzyme system, aryl hydrocarbon monooxygenase, and lung cancer. A sensitive and convenient radioactive assay for BP monooxygenase has been developed and employed in studies of kinetics, inhibition, and induction of this enzyme system and the nature of its products; and a new fluorometric assay for this enzyme has been applied to rat lung tissue for similar studies. A method for removing hemoglobin from lung microsomes by sepharose chromatography has been developed, and the spectral properties of cytochrome P-450 in these microsomes investigated. Another method has been developed which allows measurement of cytochrome P-450 in the presence of hemoglobin. The isolation and characterization of a purified preparation of lung cytochrome P-450 itself has been achieved.

Rat lung microsomes have been fractionated in a procedure involving selective aggregation of fragments of the endoplasmic reticulum with cesium ions and a pure lung microsomal fraction isolated which consists almost exclusively of rough vesicles. These rough microsomes contain about 2/3 of the activity of both NADPH-cytochrome P-450 reductase and BP monooxygenase. The products of BP metabolism by total, rough, and smooth microsomes from the liver and from the lung of rats both before and after methylcholanthrene induction have been characterized using high pressure liquid chromatography. A sensitive modified assay for epoxide hydratase has been developed and this activity characterized in lung tissue; a more sensitive assay for glutathione-S-epoxide tranferase has been developed. Levels in lung of glutathione and enzymes of its metabolism have been determined. It has been shown that lung microsomes metabolize 3-hydroxybenzo(a)pyrene to a product which binds covalently to DNA. Liver nuclei have been shown to metabolize BP to DNA binding products and these products have been identified. The nature of conjugation and its relative importance in BP metabolism by isolated, intact hepatocytes has been studied. These hepatocytes metabolize BP to DNA binding products and these products have been identified. Inhibitors of this binding have been investigated in order to evaluate the relative importance of different detoxification pathways. Human lung microsomes have been isolated and characterized in terms of their cytochrome P-450 system and metabolism of polycyclic hydrocarbons.

Since the aryl hydrocarbon monooxygenase system is an extremely complex linkage of various molecular components, the investigators at the University of Texas (Southwestern Medical School at Dallas) (N01-CP-33362) are analyzing the role of the various molecular components in lung microsomes. Several chemical and immuno-chemical inhibitors of the NADPH-cytochrome P-450 reductase-dependent reactions have been used to investigate the normal and carcinogen-induced cytochrome P-450-dependent monooxygenases in the lung. Results indicate that this enzyme is the flavoprotein reductase of cytochrome P-450 in lung hydroxylation reactions, donating at least one electron to cytochrome P-450 when either of the two reduced pyridine nucleotides NADPH or NADH is used as a source of reducing equivalents. Use of chemical inhibitors of BP hydroxylase or biphenyl hydroxylase shows that the lung contains a constitutive aromatic hydroxylase which is quite active, but does not hydroxylate BP well. A 3-methyl-cholanthrene inducible aromatic hydroxylase exists which is P-450-dependent and which appears to hydroxylate BP but does not hydroxylate biphenyl. These results indicate that two types of cytochrome P-450-dependent aromatic hydroxylases exist in lung, but only the inducible hydroxylase appears to be capable of metabolizing BP.

A cytochrome P-448-dependent substrate has been developed. This substrate, ethoxyresorufin (7-ethoxyphenoxazone), has a high degree of specificity for carcinogen-induced cytochrome P-448. The time course of induction of BP hydroxylase and ethoxyresorufin deethylase activities have similar high sensitivities to certain inhibitors such as 7,8-benzoflavone. The assay for deethylase activity is simple and very sensitive, and has already found application in assessing the state of induction of certain tissues.

Studies of BP metabolism have been initiated using the important recent application of high pressure liquid chromatography to metabolite identification. Metabolite profiles from lung and liver are investigated under varying conditions of induction and inhibition of the BP monooxygenase system. Other studies using electron spin resonance coupled with gas chromatography-mass spectrometry support the suggestion that certain BP phenols can be oxidized, either chemically or enzymatically, to yield anion products with free radical signals.

Comparison of the liver and lung BP monooxygenases of hamster, human and rat indicate that the rate of BP metabolism by human liver was only 15% of that of rodent liver while lung BP metabolism in human tissue was nearly identical with that in rodents. Content of the terminal oxidase cytochrome P-450 is very low in rodent lung and was not detectable in human lung fractions.

High pressure liquid chromatographic analysis of the BP metabolite ratio showed no statistical differences in benzo-ring product distribution (7,8- or 9,10-dihydrodiols) among rodent lung and liver or human liver. Benzo-ring products produced by human lung microsomal fractions, however, accounted for 18% of total metabolites compared to 11-12% for rodent lung fractions. Since the 7,8-diol is known to be metabolized to an

ultimate carcinogenic form of BP, the 7,8-diol-9,10-oxide, this result may have some significance in terms of increased susceptibility to BP carcinogenesis in those species (or individuals) capable of producing a larger fraction of proximal carcinogen.

It is known that lung microsomes can metabolize 3OH-BP to a product(s) which binds covalently to DNA. Extension of these studies using spectrophotometric and HPLC techniques has detected the products of BP phenol metabolism (using 9OH- as well as 3OH-BP as substrate). The steady state products appear to be diphenols (dihydroquinones).

Two projects support efforts in the critical area of establishing markers for evaluation of preneoplastic lesions in the respiratory tract. These efforts are directed at establishing objective parameters for assessing the status of preneoplastic lesions using advanced spectrophotometric and computerized procedures in cytologic evaluation.

At the Baylor College of Medicine (N01-CP-65837) optical data digitizing techniques and computerized image analysis procedures are employed to identify specific morphometric features of cells from the human respiratory tract. Stages of squamous cell carcinogenesis have been recognized and are amenable to description by statistically evaluated properties. Descriptors in this evaluation include quantifiable color changes in the cells which can be associated with different stages of carcinogenesis. Preliminary results suggest a statistically significant difference between metaplastic cells of subjects who develop dysplasia and those who do not.

At the University of Chicago (N01-CP-65777) efforts are focused upon the feasibility of assessing reversible and irreversible cell changes by means of an objective, automated pattern recognition system. The major objective is to describe markers capable of differentiating between reversible and irreversible changes in cell populations in the progression to cancer. Animal model systems for respiratory carcinogenesis are employed.

Exfoliative cells are evaluated by cytopathologists during the carcinogenic process and also by a three-color photomultiplier technique giving digitized cell images. Analysis of these digitized cell images by computer is accomplished by use of 25 cell descriptive features out of more than 200 total features which are best able to describe a cell's normal/pathological status. These features are defined by color, shape, texture, and many other morphologic parameters. Histopathologic confirmation is done on all animals at death with this study limited to animals which develop histologically-proven squamous lesions with exclusion of those developing non-squamous lesions. Results demonstrate the capability of this system to recognize and separate control cell groups from preneoplastic and from cancer cells.

Since retinoids have such a profound effect on cellular differentiation in respiratory and many other epithelia of the body, and since they have been shown to inhibit carcinogenesis in several organ systems,

the biochemical bases of these phenomena have been investigated, with the long-term goal of achieving more effective use of retinoids in cancer prevention. An intracellular retinoic acid-binding protein (RABP), which may mediate the function of retinoic acid by serving as its cellular receptor, is present in many epithelial tissues of animals (Southern Research Institute, N01-CP-22064). RABP has been found in the nuclei as well as the cytoplasm of cells of chick embryo skin and may be a membrane-bound receptor. The binding affinity of RABP for β-retinoic acid and its synthetic analogs correlates well with their biological activity, a correlation not found for binding to serum albumin, the protein involved in transport of retinoic acid and its analogs in blood. RABP has been purified, and the involvement of thiol groups in the binding of retinoic acid has been demonstrated.

In other studies it has been shown that N-ethylretinamide is tightly, but reversibly, bound to rat liver microsomes and that binding is strongly inhibited by retinol and retinoic acid, but not by retinyl acetate or methyl retinoate. This group has already shown that both lung and liver microsomes and cytosol contain esterases which cleave methyl and ethyl retinoate.

Investigations in this project (Southern Research Institute, N01-CP-22064) continue to evaluate selected retinoids for biological activity in control of epithelial differentiation. Activity of retinoids in inhibition and reversion of N-methyl-N^{1}-nitro-N-nitrosoguanidine-induced hyperplasia in organ cultures of mouse prostate is determined. In this system β-retinoic acid and several other retinoids have shown significant activity, including the 14-fluoro analog of ethyl retinoate. The capacity of retinoids to reverse testosterone-induced hyperplasia in mouse prostatic organ cultures has also been shown: β-retinoic acid, 13-cis-retinoic acid, N-ethyl-retinamide, trimethylmethoxyphenyl analogs and the 1-methoxyethyl-cyclopentenyl analog of retinoic acid are all effective. Estrone, on the other hand, had no effect on testosterone-induced lesions in these prostatic explants.

Since clinical application of retinoid chemoprevention of cancer requires detailed knowledge of retinoid toxicity, a number of investigations pursue this important aspect of retinoid anticarcinogenesis. Subchronic studies of retinoid toxicity in rats show that doses of 13-cis-retinoic acid three to five times higher than all-trans-retinoic acid are required to produce increases in concentrations of plasma alkaline phosphatase and decreases in concentrations of hemoglobin. Dose-related decreases in plasma albumin are seen in rats treated with either retinoid. Subacute toxicity studies of N-ethylretinamide, N-hydroxyethylretinamide, and N-hydroxyphenyl-retinamide in mice indicate that these compounds are not very toxic.

Since some of the common symptoms of retinoid toxicity are also seen as a result of prostaglandin activity, the interesting hypothesis is being pursued that such toxicity may be mediated by prostaglandin production. Inhibitors of prostaglandin synthesis have therefore been investigated for possible antagonism of the toxic effects of sublethal

levels of retinoic acid. Since bone thinning (resorption) and occurrence of fractures provide a dose-dependent indication of retinoid intoxication in mice, this end point has been employed. Aspirin, Ibuprofen and Indomethacin are all nonsteroidal anti-inflammatory agents which inhibit prostaglandin synthetase. Ibuprofen and Indomethacin, more potent synthetase inhibitors than aspirin, also appear to be more effective in protecting mice against bone fractures produced by sublethal retinoid intoxication.

Chemoprevention of epithelial cancer by retinoids will require compounds capable of arresting, reversing or otherwise inhibiting the carcinogenic process in the desired target organ and tissue, while at the same time providing sufficiently non-toxic effects upon the host. A new program has been initiated this year in retinoid synthesis to provide a variety of compounds for this purpose. At the University of California (N01-CP-75934) efforts will emphasize in part the synthesis of lactone derivatives of retinoids with a number of compounds already synthesized. At the Research Triangle Institute (N01-CP-75932) the focus will be upon the synthesis of derivatives of 13-cis retinoids, cyclic derivatives of all-trans retinoids, all-trans retinoids substituted in the polyene side chain and analogs of retinyl amine.

At Columbia University (N01-CP-75897) a number of retinal structures have been synthesized and their properties assessed. Methylated retinals and dihydroretinals have been found to be very unstable, unlike the dihydroretinoic acids. Efforts at the University of Houston (N01-CP-75935) are directed at the synthesis of retinoids which may be possible metabolites in living systems, and other retinoid analogues. Several of these compounds have already been synthesized and tested in organ culture assays for activity.

Four new types of retinoids having unusual shape due to their interesting 7-cis geometry have been prepared at the University of Hawaii (N01-CP-75933) prior to the current contract. These new compounds are synthesized as mixtures of geometric isomers. Efforts in this project will be directed at preparation of these compounds with efficient separation of the isomers in quantities sufficient for assay of biological potential in anticarcinogenesis. At the Stanford Research Institute (N01-CP-75931) efforts concentrate on analogs having both steric and electronic modifications in the ring, side chain and polar terminus of the retinoid skeleton. Several series of compounds have already been synthesized and are in the process of being assayed for biological activity. In all these projects great emphasis is placed upon analysis, purity and conditions for stability of these compounds prior to their entering the screening process.

Since studies of metabolism, toxicology and pharmacodynamics have been severely hampered by lack of radioactive retinoids, three new contracts were initiated this year exclusively for the synthesis, isolation and purification of these compounds. Compounds to be synthesized will depend upon Program requirements for studies of metabolism, toxicology and pharmacodynamics and may involve modifications of ring, side chain or

polar terminus with position of label and isotope varied as necessary. At the Midwest Research Institute (N01-CP-75911) procedures have been worked out for the synthesis of 12-^{3}H-cis-retinoic acid, and other procedures are presently being evaluated for the synthesis of two other radioactive retinoids. At the New England Nuclear Corporation (N01-CP-75937) several approaches to the synthesis of retinoic acid [10-^{3}H] have been evaluated as to time, labor, availability of precursors and ease of selective labelling with tritium. Two procedures which diverge from a common precursor, β-vinyl-ionol [vinyl-^{3}H] were most promising and chosen for further study. Both procedures have resulted in the desired synthesis, and resulted in the isolated, purified product. At the Stanford Research Institute (N01-CP-75936) a number of syntheses have already been completed. These include the synthesis of 5mCi of ^{14}C-labelled retinylidine-acetylacetone; the synthesis of the penultimate intermediate for the preparation of 11,12-ditritioretinol; and the conversion of unlabelled retinol to retinylidine acetylacetone. In all these projects, high requirements exist for purity, identity and stability of compounds, as well as position of radioactive label.

Retinoids have been shown to inhibit epithelial carcinogenesis in skin, mammary gland, urinary bladder and respiratory tract. This past year four new contracts have been initiated to continue and to broaden these long-term studies of prevention of epithelial cancer by retinoids. At the American Health Foundation (N01-CP-75940) a number of retinoids are being assessed for their potential to inhibit colon carcinogenesis using two known animal models: subcutaneously injected 1,2-dimethylhydrazine and N-methyl-N^1-nitro-N-nitrosoguanidine applied intrarectally, with both studies employing Fischer F344 rats. All animals have been treated in a total of 25 groups, including controls. The possible applicability of retinoid chemoprevention of carcinogenesis in this organ is of great interest in view of the high incidence of this disease among cancer sites in the United States and western world.

Two projects have been initiated to determine the relative effectiveness of different retinoids in preventing and/or reversing the carcinogenic process in the urinary bladder. At the Middlesex Hospital Medical School (N01-CP-75938) 13-cis retinoic acid is being evaluated in an ultrastructural histogenesis study to determine its effect on the urothelium of hydroxybutyl-butylnitrosamine (HOBBN)-treated Fischer F344 rats. This nitrosamine is a known selective bladder carcinogen. A life-time study of the efficacy of the retinoid retinoic acid ethylamide in inhibiting the development of bladder cancer in (HOBBN)-treated rats is also in progress. At the University of Wisconsin (N01-CP-75909) another previously developed experimental model for induction of bladder cancer with N-[4-(5-nitro-2-furyl)-2-thiazolyl]-formamide is being employed to assess the ability of 13-cis-retinoic acid in prevention of development of bladder cancer. This new project also is in its early phases with almost one thousand animals in ten groups already treated or in control groups.

At the IIT Research Institute (N01-CP-75-75939) studies are in progress assessing the chemopreventive activity of retinoids for inhibition of

development of mammary cancer. In other work (see above) this contractor has shown that retinyl acetate suppresses the appearance of mammary adenocarcinomas induced in Sprague-Dawley female rats by 1-methyl-1-nitrosourea (MNU) or by 7,12-dimethylbenzanthracene. In addition, in ongoing studies retinyl methyl ether, retinyl butyl ether and the hydrocarbon retinoid compound axerophthene suppress palpable mammary tumors induced in Sprague-Dawley female rats by MNU. This project will extend these efforts with particular emphasis upon use of retinoids with increased organotrophism for the mammary gland combined with increased chemopreventive activity and diminished toxicity.

In summary, the Lung Cancer Program is pursuing a coordinated program of studies at the human, animal, cellular, and molecular levels to investigate the factors which are involved in the cause and prevention of lung cancer. It is hoped that within this coordinated framework there will be the most rapid dissemination and utilization of new information obtained at the basic levels, and that we thus may hopefully be able to shorten the time before we achieve the desired goal of prevention of lung cancer in man.

Carcinogenesis in Human Tissues

Parallel to efforts in the Carcinogenesis Program, which have developed experimental animal models of epithelial cancer for many primary targets of human carcinogenesis, has been the important development of the use of human tissues for studies of carcinogenesis in several organ systems at high risk for cancer in man. These studies in organ and cell culture, as well as in xenotransplanted specimens to immunologically-deficient animals, importantly complement those in experimental animal models and, in fact, constitute the only feasible nonepidemiological approach presently known for wide-ranging carcinogenesis investigations in human tissues. This parallel approach using human model systems for studying carcinogenesis in target tissues of man represents a unique opportunity for linking investigations using experimental animals with human cancer.

This past year this program has received increased emphasis with the initiation of four new contracts for studies of carcinogenesis in human tissues of the bladder, prostate, endometrium and esophagus.

At the University of Kentucky (N01-CP-75954) efforts will be devoted to development of cell and organ culture procedures for maintenance of human bladder and prostatic epithelial tissue in vitro, and to the transformation of these tissues by chemical carcinogens. Early work has concentrated on studies of bladder and development of procedures for obtaining high yields of cells and exploring the use of several culture media. Data to date indicate that CMRL 1066 or S77 media supplemented with ten percent fetal calf serum is satisfactory.

At the Middlesex Hospital Medical School (N01-CP-75955) the objectives are to obtain viable normal tissue, with intact urothelium supported by

mesenchyme, from human bladders; to establish long-term organ and cell cultures from this material; to determine the parameters for normal growth of the normal human urothelium in vitro; and to conduct carcinogenesis studies using these human bladder explants. Early efforts in this new project have emphasized culture media investigations, including Waymouth's medium MB 752/1 and Ham's medium with and without supplements. Use of glycine solutions for irrigation of tumor-bearing bladders before biopsy appears to result in much healthier appearing urothelia than previous surgeon practice of distilled water

At the University of Maryland (N01-CP-75909) studies of human esophageal tissue with similar objectives are underway with especial current emphasis on ultrastructural morphological investigations. High resolution light, scanning and transmission electron microscopy are employed. Baseline studies of squamous cell carcinoma of the esophagus show clusters of tumor cells outlined by basal lamina which was not seen in between the cells. Villous cytoplasmic processes projected into the intercellular spaces but true lumen was not found. Abundant tonofilaments and dispersed fine filaments were present; most nuclei were pleomorphic, and prominent nucleoli were seen. Ultrastructural studies are also in progress on normal esophageal tissue which has been maintained in organ culture in some instances for as long as six months.

In the fourth new contract at the University of North Carolina (N01-CP-75956) objectives will focus upon the study of human endometrial tissue and its interactions with chemical carcinogens to determine whether specific insights can be obtained into the causation of endometrial carcinoma. Early efforts have emphasized acquisition of appropriate human tissue, and the maintenance of this tissue in culture. A variety of cell and organ culture techniques as well as tissue culture media have been explored. Results have been very promising with maintenance of organ cultures in a variety of media including one which is chemically-defined and lacking serum supplementation. Some cultures have been maintained as long as four months with good preservation of epithelial morphology. Identification of cells in culture is studied and relation to intrinsic endometrial cell populations determined, as well as effects of hormone supplementation upon the functional capacities of these cells in culture.

Studies at the University of Maryland (N01-CP-43237) continue to provide a wealth of information on normal, preneoplastic and malignant human tissues. Bronchial epithelium, pancreas, breast and colon are investigated morphologically and cytochemically before, during and after growth in explant culture. Tissue responses to carcinogen applications in culture are studied. Clinical histories and a patient questionnaire are used so that possible correlations can be made in the future. Quantitative studies of carcinogen binding to ostensibly normal bronchial epithelium are in progress with correlative efforts relating binding to histogenetic tumor type and epidemiological data.

Cultures of bronchial epithelium have been exposed to benzo(a)pyrene-ferric oxide over a two-month period. Such explants show epidermoid metaplasia

and lesions resembling carcinoma in situ. Over one hundred and twenty human primary lung carcinomas have been examined ultrastructurally and histochemically and classified according to new histogenetic criteria developed.

Studies of human pancreatic ductal epithelium show this human tissue has the capacity to activate the polynuclear aromatic hydrocarbons benzo(a)-pyrene and 7,12-dimethylbenzanthracene to metabolic intermediates which bind to DNA. Cytological changes at the light and electron microscopic levels are produced in explant cultures by the direct acting carcinogen N-methyl-N[1]-nitro-N-nitrosoguanidine which closely resemble those occurring in human metastasizing adenocarcinomas and in the Syrian hamster model of pancreatic carcinoma.

Morphological examination of normal, dysplastic, premalignant and malignant human breast tissues continues with over ninety-nine cases now available for study. Normal tissue can be easily maintained in explant culture for 3 to 6 months and shows characteristics of adeno and epidermoid differentiation. Treatment of normal epithelium with the carcinogens DMBA and MNNG results in hyperplastic areas, with cells showing high nuclear to cytoplasmic ratios, aberrant nucleoli and marked scalloping of the ductule on the basal surface. Cells in the outgrowth from these explants show random orientation and adeno and epidermoid characteristics. Similar studies with human colonic epithelium progress with over one hundred twenty-seven cases available for study.

The Veterans Administration Hospital (Washington, DC) (Y01-CP-60204) continues to provide human respiratory tract tissues for studies and characterization in vitro of normal, premalignant and malignant epithelium. Such studies include the response of these tissues to carcinogens in culture and as xenotransplants. In all cases detailed abstracts of patient clinical records are prepared, including gross pathology encountered at surgery and pertinent aspects of operative procedure employed. Tissues are prepared for ultramicrotomy and electron microscopy and are characterized by these procedures and by high resolution light microscopy.

Studies of carcinogenesis in human tissues at Litton-Bionetics, Inc. (N01-CP-43274) utilize the athymic nude mouse as host animal for xenotransplanted human tissues previously exposed to carcinogens in culture, or exposed to carcinogens after xenotransplantation is completed. The establishment of an animal host for human tissue xenografts treated with carcinogens or anti-carcinogens may provide a model more predictive of the effects of carcinogens/anticarcinogens on human tissues than extrapolation from an animal tumor or tissue culture system. A barrier-sustained specific pathogen-free facility has been developed. This facility has proved adequate to breed and maintain the athymic nude mouse for long-term studies. In this environment a normal life span of over two years has been achieved. It has been established that the nude mouse will support long-term survival of human bronchial tissue xenografts, maintaining graft viability and normal morphology of over 400 days. Human bronchial segments, human colon, human esophagus, human

pancreatic duct and bovine pancreatic duct tissues are presently under study.

Two new contracts have been established this year for the isolation, identification and culture of epithelial cell types from the colon of the rat and the bovine pancreatic duct. Methods developed in these animal systems may also prove applicable to (corresponding) human organ types. At the Southern Research Institute (N01-CP-75914) early work has shown that repeated, timed enzymatic dissociations of colonic epithelium with trypsin-DNase results in a gradual dissociation of the mucosa giving rise to preparations containing essentially differentiated cells on the one hand and preparations containing essentially proliferating cells on the other. Identification of the proliferating cells was made by pulse-labeling the rat with tritiated thymidine two hours prior to sacrifice and the following sequential enzyme dissociations of the colonic mucosa. Differences are also seen by scanning electron microscopy in the surface characteristics of these cell preparations. At the University of Maryland (N01-CP-75947), a new method for isolating pancreatic ductal epithelial cells has been developed which enables these cells to be separated free from underlying stroma and almost entirely free of fibroblasts. The method is based on a new method of digestion of perfused whole bovine pancreas allowing flotation of sheets of cells rather than more complete digestion to individual cells used in the past. These isolated pancreatic ductal cells can be maintained in culture for at least two months. Efforts are directed at the light and electron microscopic characterization of these cells as well as characterization of antisera for these cells by immunofluorescence and immunoperoxidase techniques.

Pancreas Carcinogenesis

As previously reported, the pancreas carcinogenesis program had successfully achieved one of its principal, initial objectives with the development of three potential animal models: 1) methylnitrosourea induction in strain 13 guinea pigs, 2) azaserine in rats, and 3) N-nitrosobis (2-hydroxy)propylamine in Syrian golden hamsters (the latter obtained in the course of a systematic bioassay and study of substituted nitrosamines). These results justified further exploration in each of these models, the last model in particular showing high promise and considerable extension by program and the scientific community. Other studies were initiated based upon two hypotheses for the mechanism by which a carcinogen can come in contact with the susceptible pancreatic cell: 1) preferential uptake from blood, concentration in pancreatic tissue and excretion through the pancreas ductules, and 2) excretion of conjugated carcinogens in bile through the common bile duct followed by reflux or reverse flow of pancreatic juice and bile constituents into the head of the pancreas where deconjugation could release the carcinogen at the site of the susceptible cell. Each of these aspects continues to be explored with the acquisition of important baseline data and the attainment of some very interesting results.

a. Human Studies - In the contract at the Mayo Foundation (N01-CP-55660) one objective has been to determine the anatomical relationships of the human pancreatic duct, bile duct and duodenum and to relate these anatomical arrangements to duct cell histology. Information was also obtained regarding smoking and alcohol habits, serum triglycerides, cholesterol, occupation, sex, age, cause of death and other factors. Analyses of autopsies completed on 265 out of 330 cases indicate that the incidence of abnormal histology is related to increasing age, respiratory disease (chronic obstructive pulmonary disease and pneumonia) and neoplastic causes of death. A trend was also noted toward increased frequency of abnormal duct histology in those postmortem specimens with separate openings of the common bile duct and pancreatic duct compared to other ductal entries into the duodenum. In this regard, these data confirm the very recent report from the Memorial Hospital. More data and analysis are needed to establish firm statistical associations among the many variables under study though preliminary suggestions are that a combination of factors may be related to induction of pancreatic neoplasia. In this regard, an analysis of multiple variables presently underway should prove very interesting, including cigarette smoking and use of alcohol and their significance to the production of abnormal pancreatic duct histology.

A second major effort in this contract has resulted in the development of a canine model for induction of pancreatic duct reflux. This model allows simultaneous quantification of pancreatic enzyme outputs, duodenal volume flow, and pancreatic and duodenal pressures under physiologic conditions. The interesting observations were made that although mean fasting pancreatic pressure exceeded mean fasting duodenal pressure, both pressures increased shortly after ingestion of a meal such that at twenty minutes after eating, the pressure relationship was reversed, with mean duodenal pressure exceeding mean pancreatic pressure for a twenty minute time duration. Further, elevation of postprandial pancreatic pressure occurred concomitantly with increased pancreatic enzyme output and the increased duodenal pressure with increased duodenal volume flow. These circumstances are postulated to favor reflux of duodenal contents into the pancreatic duct, and indeed reflux of a small amount of duodenal contents has been observed.

More recent studies demonstrate that the hormone secretin alters pressure relationships between the pancreatic duct and duodenum, demonstrating that hormones have the potential for influencing reflux of duodenal contents into the pancreatic duct. Other gastrointestinal hormones and drugs, particularly nicotine, will be studied for their possible effect upon reflux. It is important to note that if separate openings of the pancreatic duct and bile duct into the duodenum (rather than a common entry) do develop as an underlying significant feature in pancreatic carcinogenesis, then if reflux is important, contents refluxed from the duodenum itself rather than just bile may be a possible mechanism for induction of pancreatic cancer.

b. In Vivo Studies - In vivo studies of pancreatic carcinogenesis continue in several animal species so as to provide program with a better understanding of model systems. The azaserine rat model (Dartmouth Medical School, N01-CP-33378) has been extended to nitroso derivatives of amino acids selected on the basis of tissue localization and mutagenicity tests. Studies with the methylnitrosourea derivatives of lysine, ornithine and diaminobutyric acid indicate that these compounds are more effective as carcinogens in breast, skin and kidney than in pancreas. The lysine derivative appears to be especially effective as a renal carcinogen, whereas the ornithine compound is most effective as a skin and breast carcinogen. All three of these compounds cause a high incidence of atypical acinar cell nodules in the pancreas.

In the contract at Northwestern University (N01-CP-75876) studies continue on the histogenesis of guinea pig pancreatic adenocarcinoma. Pancreatic adenocarcinomas have been found in 29% of inbred strain 13 animals between 28 and 44 weeks of N-Methyl-N-Nitrosourea treatment. Histogenetic studies strongly suggest that these MNU-induced adenocarcinomas are derived from transformation of acinar cells. On the other hand, treatment with azaserine for 24 weeks has shown neither atypical acinar cell nodules nor carcinoma, while treatment with yet a third compound, 2,2-dihydroxy-di-n-propyl-nitrosamine, for 30 weeks has produced angiosarcomas of the liver in nearly 75% of the guinea pigs, but only a single (acinar cell) carcinoma of the pancreas.

At the University of Utah (N01-CP-55709) a primate model has been developed for assessing biliary pancreatic reflux and its possible significance for human pancreas cancer. These studies which identify appropriate ductal pressure conditions provide the first quantitative demonstration of bile reflux under relatively physiologic conditions through the pancreatic duct of monkey, a primate that has a biliary-pancreatic ductal system similar to man.

In conjunction with the primate reflux studies, a second phase of investigation has involved evaluation of the physiology and pharmacology of the sphincter of Oddi in the opossum. The opossum provides an ideal animal model in that its easily accessible extraduodenal sphincter exhibits spontaneous electrical activity and simultaneous rhythmic contractions. In this model, pressure, flow, and electrical activity have been recorded simultaneously before and after the administration of test hormones or drugs. It has been found that the electrical activity of the opossum sphincter of Oddi precedes its mechanical activity and correlates directly with it. This is manifested by decreased ductal flow, increased ductal pressure, and increased frequency of bursts of spike potentials at the time of visible papillary muscle contraction. This electrical pattern is distinct from duodenal activity. Cholecystokinin (CCK), CCK-octapeptide, and pentagastrin all effect a nearly simultaneous increase in sphincter electrical activity and common duct (CD) pressure with a decrease in CD flow. This action is independent of gallbladder contraction. Secretin has little effect on biliary kinetics. Dose-response studies with CCK, octapeptide, and pentagastrin show a direct

relationship between dose and response with sphincteric contractile activity at all dose levels. Early studies have revealed a similar pharmacologic response to CCK and pentagastrin in the monkey biliary system.

These reflux studies in the Rhesus monkey lend credibility to the concept that bile-born carcinogens might gain ready access to the proximal pancreatic duct, while the opossum data support recent studies which refute the classical concept that CCK relaxes the sphincter of Oddi while causing the gallbladder to contract. These data also clearly demonstrate the contractile effect of pentagastrin on the distal biliary sphincter, suggesting that these hormones may act in harmony to modulate bile flow.

At the University of Illinois Medical Center (N01-CP-65843) studies designed to investigate the carcinogenic potential in F_1 progeny of Hartley guinea pigs of maternal transplacental exposure to N-nitrosomethylurea (NMU) and N-nitrosomethyl-urethane (NMUT) indicate that no tumors occur which are related to carcinogen treatment. A similar conclusion has been drawn from a study testing the carcinogenic effects of these agents in guinea pigs following partial pancreatectomy.

Efforts at the University of Nebraska, Eppley Institute for Research on Cancer (N01-CP-33278) continue to develop the hamster-nitrosamine model for pancreatic carcinogenesis which originated from this institute. It has now been shown that a single injection of N-nitrosobis (2-oxopropyl)-amine (BOP) to Syrian golden hamsters results in the selective induction of pancreatic cancer. This single dose protocol shows an excellent dose/response relationship and should be very valuable in future studies of pancreatic carcinogenesis.

The effect of cholecystoduodenostomy and choledochostomy upon pancreatic carcinogenesis induced by BOP in hamsters appears to be negligible since induced lesions were similar in morphology, multiplicity and distribution to those in animals without surgery. These findings contraindicate the importance of biliary reflux in pancreatic tumor induction in this model.

A new contract has been initiated this year to explore the potential of the European hamster as a model for pancreatic carcinogenesis studies. At the Medizinische Hochschule Hannover (N01-CP-75972) range-finding studies have been initiated using the proven pancreatic carcinogen 2,6-dimethyl-nitrosomorpholine, and future studies will employ the related compound 2,6-dimethyl-nitrosopiperazine.

c. Biochemical and Pharmacologic Studies - Projects at Indiana University (N01-CP-55654) and Dartmouth Medical School (N01-CP-55708) were established to examine uptake, retention, and excretion of a variety of chemical moieties of varying structures. Knowledge of this relationship may contribute to an understanding of organ specificity of pancreatic carcinogens and potential neoplastic agents. It is already

well known that amino acids are relatively rapidly absorbed from the blood stream, metabolized in the synthesis of proteins and excreted by the exocrine and endocrine physiological functions of the pancreas. Continuing studies show that several steroid hormones, including estriol, estradiol and progesterone have shown the greatest tissue: plasma ratios, second only to certain amino acids. Retention of these hormones is poor, and the decay curves are more rapid in the pancreas than in the plasma.

At the University of Indiana uptake, retention and excretion of a variety of compounds is investigated in order to determine chemical and structural parameters which may be required for these physiological processes to occur in the pancreas. Modest uptake has been shown in the rat pancreas for the acidic compounds benzoic acid, benzo(b)thienyl-3-acetic acid and salicylic acid. Retention of the latter two compounds to some extent appears to occur; chemical tests for benzoic acid, however, indicate no significant retention occurs, suggesting radiolabel retained represents metabolite(s). A variety of basic compounds, including decamethonium, isoniazid, D- and L-methadone and propranolol, show significant pancreatic uptake. Of these, L-methadone and propranolol show a greater tendency for pancreatic retention than their metabolites. Half-lives in plasma, pancreas and pancreatic secretions have been determined for decamethonium, D-amphetamine, d-methadone, L-methadone and propranolol. Compared to the half-life in plasma these measurements indicate that the pancreas acts as a selective filter for these basic compounds. In addition, low doses of propranolol were shown to reduce pancreatic secretory activity.

At the Dartmouth Medical School (NO1-CP-55708) radiochemical tracer techniques have been used to study the distribution of sixty-eight compounds into plasma, pancreas, liver and kidney of rats and hamsters. In these studies evidence for excretion into the pancreatic duct system has been sought. Active uptake by the pancreas has been found for a variety of alpha amino acids. The pancreas appears to show a low degree of structural selectivity in this uptake of alpha amino acids, provided both free amino and carboxyl groups are present. Acylation or dialkylation of the amino group or esterification of the carboxyl group abolishes active uptake, while monoalkylation on nitrogen or on the alpha carbon atom has little effect. Both D and L isomers are taken up and to a similar extent. Studies with labeled polycyclic aromatic hydrocarbons have shown an initial rapid uptake of radioactivity by the pancreas, followed by a slower wash out. Of other compounds known or suspected to be pancreatic carcinogens (NMU, AAF, DAB, azaserine, benzidine, and BHP) only azaserine appears to have a marked affinity for the pancreas. However, animals receiving NMU excrete considerable radioactivity into the pancreatic duct system. The nature of this activity is not known.

At the Medical College of Georgia (NO1-CP-55656) the metabolic capacity of the rat pancreas is explored for biotransformation of chemical carcinogens and other agents. The three polycyclic aromatic hydrocarbons, 3-methyl-

cholanthrene (3MC), dimethylbenzanthracene (DMBA) and benzo(a)pyrene (BP) have been studied. High pressure liquid chromatographic analysis of pancreatic metabolites of 3MC shows that hydroxylation occurs at both the 1,2-position and the 11,12-position with the 1,2-position the major pathway. Hepatic metabolism shows similar products. Analysis by HPLC of DMBA and BP metabolism are in progress. The pancreatic metabolism of BP is enhanced by pretreatment with both 3MC and phenobarbital, in contrast to that of 3MC and DMBA.

Metabolic studies of the hamster pancreatic carcinogens N-nitrosobis (2-hydroxy)propylamine (BHP) and N-nitrosobis (oxopropyl)amine (BOP) at the University of Nebraska, Eppley Institute for Research on Cancer (NO1-CP-33678) continue efforts to elucidate the organotropic carcinogenic effects of these compounds on the hamster pancreas. Carbon-14 labeled BHP and BOP were found to be extensively metabolized in hamsters to labeled carbon dioxide, and metabolism of each of the carcinogens results in unidentified nitrosamine metabolites in the urine, as well as N-nitroso-(2-hydroxypropyl) (2-oxopropyl)amine. In other studies, quantitative differences in BOP and BHP metabolism have been found between the hamster and the rat. These compounds are not pancreatic carcinogens in the rat.

Biochemical studies at the University of Illinois (NO1-CP-65843) have shown DNA damage and repair in the pancreas of Hartley strain guinea pigs following carcinogen administration. Damage and repair caused by methylnitrosourethane and 4-hydroxyaminoquinoline-1-oxide, both in vitro in pancreatic slices and in vivo following a single injection, were assayed on alkaline sucrose gradients using preparations of isolated pancreatic nuclei. Under the conditions used, in vitro damage sustained within 30 minutes of exposure was repaired within two to three hours, while damage sustained in vivo was gradually repaired over a one to two week period, in liver and pancreas respectively.

d. <u>In Vitro Studies</u> - The potential advantages of in vitro systems for investigations of pancreatic carcinogenesis are being explored in two contracts. These efforts are entirely devoted to determination of necessary procedures and methods for handling and maintaining pancreas tissues in vitro. The contract at the University of Maryland (NO1-CP-75947) is discussed under the section on Carcinogenesis in Human Tissues.

At the American Type Culture Collection (NO1-CP-65751), studies to establish reproducible procedures continue for isolation, maintenance, and identification of pancreatic duct and exocrine cells. Results indicate that conditioned media from some primary and established cell lines are capable in short-term experiments of stimulating proliferation of pancreatic exocrine cells maintained as colonial aggregates in vitro.

Conditioned media from human lung fibroblast strains WI-38 and MRC 5 were most consistently effective. Further experiments have used x-irradiated lung fibroblasts as feeder layers for acinar epithelia. Marked stimulation was observed in morphological and biochemical studies, with proliferation of epithelial cells continuing even after seven weeks in culture while controls degenerate after twenty days.

In other experiments epithelia from pancreatic ducts of Syrian hamsters have been maintained in explant culture for over six weeks; and ductal epithelia from guinea pig pancreatic tissue have been isolated by dissociation of the luminal layer, and the cells retained in culture as colonial aggregates.

Mammary Gland Carcinogenesis

Efforts in mammary gland carcinogenesis in the Biology Operational Unit are focused upon synergism between estrogen and radiation in the induction of mammary gland tumors, upon organ culture studies and on possible differences in hormonal patterns among women with different risk for breast cancer.

At the Brookhaven National Laboratory (DOE-NCI Interagency Agreement, Y01-CP-30213), the reported synergistic interaction between diethylstilbestrol (DES) and x-rays on mammary adenocarcinoma formation in AxC female rats has been found to hold also for DES and neutrons. This synergism does not obtain when female rats of the Sprague-Dawley strain are studied. DES has been found to induce much higher levels of serum prolactin in AxC rats than in Sprague-Dawley rats. Thus, prolactin appears to be implicated in the synergistic interaction of DES and radiation on mammary adenocarcinoma formation. As the dose of DES is lowered in AxC rats synergism with radiation is delayed and may even be absent. The interesting finding has also been made that when the time between neutron radiation and DES treatment is lengthened, synergism of mammary adenocarcinoma formation is maintained, suggesting that there is little or no recovery or repair of neutron radiation injury.

At the Organization for Health Research (N01-CP-33330) the possible synergistic interaction of estradiol-17β (E_2) and irradiation for mammary tumorigenesis is being investigated in three rat strains of expected different susceptibilities. Irradiation is at three different dose levels with both x-rays and with neutrons of three different energies. For each type of radiation and for each dose level, four animal subgroups are studied: intact females, intact females treated with estrogen, hystero-ovariectomized females and hystero-overiectomized females with estrogen. This large study is still in progress. Results to date indicate that: the majority of all mammary tumors are benign, the most frequent type being the fibroadenoma; tumor prevalence is considerably lower in hystero-ovariectomized animals than in controls; tumors occur earlier in hormone-treated animals than in nonhormone-treated animals, both in control groups and those receiving radiation; and the Relative Biological Effect of 0.5 MeV neutrons appears to be several times greater than for 15 MeV neutrons in the rat strain of intermediate susceptibility.

Two projects examine possible synergistic effects of repeated low-dose irradiation on mammary gland carcinogenesis in estrogenized rats. These projects may provide information regarding risk for breast cancer induction from repeated mammographic examinations. Both the project at the Alton Ochsner Medical Foundation (N01-CP-65764) and that at Brookhaven

National Laboratory (Y01-CP-60219) are long-term studies which investigate several pertinent parameters such as hormonal state and obesity as well as the effects of fractionation and protraction of the x-ray dose levels employed.

At the University of Nebraska, Lincoln (N01-CP-33289), emphasis is upon the induction in organ culture of nodule-like alveolar lesions (NLAL) and upon the determination of the biological characteristics of these lesions. Biochemical characterization of these cultured mouse mammary glands is also pursued. It has been found that the whole 2nd thoracic mammary gland of 3-4 week old female Balb/c mouse is induced to pregnancy-like alveolar development after 9 days of cultivation in a chemically defined culture containing the hormones insulin (I), prolactin (Prl), aldosterone (A) and cortisol (F). Treatment of the gland with DMBA for a 24-hr. period between 3 and 4 days of culture induces NLAL in 80% of the gland and these lesions become visible when normal alveoli are allowed to regress in medium with (I + A). Less potent hydrocarbons, dibenzanthracene, benzanthracene and anthracene under similar culture conditions exhibit markedly reduced NLAL-inducing action. Biochemical studies have shown that labeled DMBA binds to purified DNA of mammary cells in culture, and that DMBA is capable of inducing aryl hydrocarbon hydroxylase in these cells. The biological potential of the NLAL and/or latent transformed cells possibly also present is now under investigation.

At the Frederick Cancer Research Center (Litton Bionetics, Inc.; Contract Number-N01-CP-75380) efforts are concentrated upon DMBA carcinogenesis and steroid mechanisms of action in the rat mammary gland. Epithelial elements have been isolated from the mammary fat pad of Sprague-Dawley rats and cultured in vitro. Their requirements for specific hormones at physiological levels to insure normal growth and morphology have been determined. A method for routine isolation and growth of large numbers of normal breast epithelial cells has been developed using collagenase dissociation of human breast tissue which results in a cell mixture of enriched epithelial elements. These cultures grow and maintain normal morphology under the influence of physiological levels of specific hormones. Metabolism of DMBA by rat and human mammory gland and epithelial cells has identified certain metabolites, and studies of DMBA metabolite binding to DNA in in vivo studies indicate greater binding to mammary gland DNA than to liver DNA at all ages of rats employed, and further that such binding was maximal at the age of maximal susceptibility.

At the University of Minnesota (N01-CP-55702) high and low risk populations for breast cancer are being studied for possible differences in hormonal and/or other physiologic patterns. A major effort focuses upon possible differences in the rhythmic changes in circulating blood levels and in urine levels of a number of hormones. In these studies plasma hormonal levels are determined in samples obtained every 20 minutes for 24 hours at different seasons of the year. It has been found that plasma cortisol and prolactin exhibit a statistically significant circadian rhythm in most subjects. Further, multivariate analysis shows that circadian rhythm characteristics differ for plasma prolactin concentration

changes between low-risk Japanese women and high-risk Minnesotan women, irrespective of age. This analysis uses data obtained to date at two seasons of the year. Such a difference between populations was not found for rhythm characteristics of thyroxine. Studies on urine levels have yet to be completed, as do many other plasma hormone variations for all four seasons of the year.

Perinatal Carcinogenesis

Tissue exposure to carcinogens during perinatal development may have significant carcinogenic consequences, some of which may not become evident until the post-pubertal and adult periods of life. Three contracts in the Biology Operational Unit investigate various aspects of this important area. At the University of Colorado (NO1-CP-75875), tissue interactions in induction and perpetuation of hormonally-induced permanent cellular alterations are under investigation. Tryptic separation and recombination methods with ultrastructural, immunocytochemical, and other studies are utilized to determine interaction mechanisms in normal, abnormal, and neoplastic growth and differentiation of the vagina. Ultrastructural data corroborate the findings of other investigators that induction of alterations in vaginal cytodifferentiation by neonatal exposure to estradiol may occur through a mechanism of vaginal epithelial cell selection.

Development of the uterus, vagina, cervix, and prostate is known to require an interaction between urogenital epithelium and its subadjacent mesenchyme which specifies the morphogenetic pattern of the epithelium. Similarly, development and expression of estrogen-induced irreversible effects in the developing vagina (ovary independent persistent vaginal cornification) requires an interaction between epithelium and stroma in which the stroma induces vaginal epithelial hyperplasia. The mechanism of these interactions may involve the transfer of macromolecules, because it appears that basal vaginal epithelial cells transport basal laminar material in a directionally-specific manner which corresponds to the known direction of inductive effects; epithelial cells endocytose and exocytose this material.

At the Aichi Cancer Center Research Institute (NO1-CP-55650) investigations are pursued on induced perinatal alterations and their influence on the carcinogenic process. The investigations are directed at the nature of permanent alterations of the vagina in mice and the coagulating gland in rats resulting from perinatal exposure to steroid hormones, and at their relation to carcinogenesis. A number of interesting findings have been made: a) Fetal vagina of the mouse is more susceptible than neonatal vagina in terms of development of irreversible alterations induced by estradiol-17β and testosterone propionate; b) hypothalamic function is apparently more sensitive to steroids at early post-natal ages; c) the vagina of neonatally-estrogenized mice may consist of mixed populations of at least two types of cells, estrogen-responsive and non-responsive; and d) irreversible vaginal changes can be induced during adult life by an additional E_2 treatment if the vagina had been primed with small amounts of E_2 neonatally.

Other investigations in this contract explore the nature of the development of ovarian dysgenesis (OD) after neonatal thymectomy (Tx). It has been found that a) sera of neonatally Tx mice with OD contain antibodies against ooplasms, and b) spleen cells taken from Tx mice with OD can regularly induce typical OD when injected into isogeneic infant mice. This suggests an autoimmune etiology of OD in these experiments.

The development of a nonhuman primate model to demonstrate transplacental chemical carcinogenesis continues at Meloy Laboratories, Inc. (NO1-CP-55613). Earlier findings have shown that 1-ethyl-1-nitrosourea (ENU) produces lytic damage to neuronal and glial precursor cells of the subependymal germinal layers of the cerebral hemispheres in fetal monkeys. Three other age groups of patas monkeys have now developed tumors following exposure to ENU. These tumors appear shortly after birth and resemble those associated with pediatric neoplasms in man. Both these characteristics of the nonhuman primate model differ from rodent models in which transplacental exposure to carcinogen results in tumor development in young adult life of tumors predominantly of adult morphology. Results with ENU exposure to pregnant monkeys indicate that the period of maximal fetal susceptibility appears to be early in gestation.

Resources and Other Projects

A number of resource contracts provide capabilities to the carcinogenesis research collaborative program. Two projects provide vital microscopic and autoradiographic technology for a variety of ongoing studies (Litton Bionetics, Inc. NO1-CP-65847; Experimental Pathology Laboratories, Inc. NO1-CP-65763). Animal breeding at Harlan Industries, Inc., (NO1-CP-55647) as a resource to the prostate program is discussed under the Interdisciplinary Program and Resources Operational Unit. A major resource for laboratory services is provided by Microbiological Associates, Inc. (NO1-CP-02199); a large number of individual experimental protocols designed jointly by NCI and the contractor have been employed in collaborative studies during the past year.

At the University of Toronto (NO1-CP-75879) the biochemical and morphological components of hepatic carcinogenesis are investigated with major focus on the purification and characterization of a component in the endoplasmic reticulum of preneoplastic hepatocytes called Preneoplastic Antigen (PN-antigen). This component seems to be special to this population. PN-antigen has been characterized as a glycoprotein of molecular 140,000 daltons, with two identical subunits each having serine as amino terminal amino acid.

Major attention in this project is directed to a new approach to the analysis of the sequence of steps in chemical carcinogenesis in vivo. The first biological step appears to be the induction by a carcinogen of random rare hepatocytes that are resistant to the inhibitory effect of carcinogens on cell proliferation. These putative initiated hepatocytes can now be assayed in vivo and in vitro. The initiation process is a two-step one in which a round of cell proliferation is required

as the second step, following the induction of one or more molecular events. In the in vivo assay, the initiated cells can be induced to proliferate very rapidly without proliferation of the surrounding liver cells and thus can be visualized within a matter of days. The use of the relatively specific marker for these cells, gamma-glutamyl transpeptidase, facilitates quantitation both of the number of foci of resistant liver cells and the number of such cells in the whole liver.

Carcinogens not carcinogenic for the adult rat liver, such as 3-methylcholanthrene,7,12-dimethylbenzanthracene, N-methyl-N^1-nitro-nitrosoguanidine (MNNG) and N-methylnitrosourea, as well as many liver carcinogens, are all positive with this new assay. Several noncarcinogenic hepatotoxins are negative.

At the Children Hospital of Los Angeles (N01-CP-55641) efforts are devoted to fibrinolysis as a parameter of in vitro transformation. The main objective of this contract is to determine the association of increased plasminogen activator production with malignant transformation and to determine whether an increased production of this protease is an early marker of transformation. In addition, the subcellular localization of plasminogen activator is being sought as well as whether increased plasminogen activator is an expression of invasive potential of certain cells.

A number of interesting findings have been made: 1) In collaboration with investigators at the NCI, it has been demonstrated that transformed mouse epidermal cells produce high levels of plasminogen activator. The increase of this protease during malignant transformation seems to appear concurrently with the ability to grow in soft agar but before the capability to grow as tumors in immunosuppressed animals. 2) It has been found that endothelial cells derived from the aorta of newborn calves have high fibrinolytic activity. These normal cells, which can invade tissue upon an appropriate stimulus, grow in soft agar and in the presence of a specific serum show morphological changes usually observed in transformed cells. They remain diploid, however, and do not grow as tumors in the <u>nude</u> (athymic) mice. The plasminogen activator secreted by these endothelial cells has been isolated and purified. 3) The cell cycle specificity of plasminogen activator production in malignant cells has been shown to be different from normal endothelial cells.

The project at Biotech Research Laboratories, Inc., (N01-CP-55640) seeks to establish criteria for in vitro carcinogenesis endpoints which correlate with tumorigenic potential. This work has resulted in the development of a cell aggregation assay to assess the transformed status of a cell population; it has been found that the survival ability of some transformed cells in the aggregate form correlates with colony formation in soft agar and with tumorigenicity in nude mice and syngeneic hosts.

CONTRACT NARRATIVES

BIOLOGY OPERATIONAL UNIT

October 1, 1977 through September 30, 1978

AICHI CANCER CENTER RESEARCH INSTITUTE (N01-CP-55650)

Title: Induced Perinatal Alterations and Their Influence on Carcinogenesis

Contractor's Project Director: Dr. Yasuaki Nishizuka

Project Officer (NCI): Dr. Jerry M. Rice

Objectives: (1) To study the nature of permanent alterations of the vagina in mice and the coagulating gland in rats resulting from perinatal exposure to steroid hormones, and their relation to carcinogenesis during later adult life; and (2) to study the mechanism of ovarian dysgenesis and ovarian tumorigenesis resulting from neonatal thymectomy in the mouse.

Major Findings: Objective (1): (a) Fetal vagina of the mouse, especially Day 17, is more susceptible than neonatal vagina in terms of development of irreversible alterations induced by both estradiol-17β (E_2) and testosterone propionate; and hypothalamic function is apparently more sensitive to steroids at early postnatal ages; (b) It appears that the vagina of neonatally estrogenized mice may consist of mixed populations of at least two types of cells, estrogen responsive and non-responsive. This was revealed by electron microscopic (EM) features of vaginal basal layers and by responses to E_2 stimulation at adult ages; and (c) Irreversible vaginal changes can be induced during adult life by an additional E treatment when the vagina was primed with small amounts of E_2 neonatally. These data suggest that cellular responses to steroids gradually decrease in grade and intensity as the perinatal period progresses, and that this is related to morphological and developmental processes of the vagina. Long-term observation of irreversible hyperplastic changes in the coagulating gland (CG) and its neighboring regions are under progress in experiments with estrogenized Wistar rats with and without additional treatment with small doses of a potent carcinogen, 4-butyl-N-(4-hydroxybutyl)-nitrosamine.

Objective (2): As for the nature of ovarian dysgenesis (OD) after neonatal thymectomy (Tx), its autoimmune etiology is strongly suggested from the following data: (a) Sera of neonatally Tx mice with OD contained antibodies against ooplasms. This was demonstrated by indirect immunofluorescence and horseradish peroxidase immuno-histochemical techniques; (b) When spleen cells taken from Tx mice with OD were injected ip into isogeneic infant mice, typical OD was regularly induced. Hence, OD could be referred to as autoimmune oophoritis. Oophoritis with similar morphology accompanied with appearance of autoantibodies, was successfully induced in infant or young adult athymic BALB/c nude mice by injection of small

numbers of spleen cells 10^5-10^6). Hormonal analysis and histogenesis of ovarian tumors from autoimmune oophoritis are under progress.

Significance to Biomedical Research and the Program of the Institute: The animal models for perinatal hormone-mediated carcinogenesis provide the best prospect for predicting the long-term effects in both sexes of perinatal human exposure to DES and other steroids. The ovarian tumor model with Tx mice is a unique system for exploration of the relation of aberrant lymphoid maturation and subsequent autoimmune diseases and neoplasia.

Proposed Course: (1) In order to understand the histogenesis and cytodynamics of the irreversible tissue alternations and their neoplastic conversions in the vagina and CG, studies with EM and histochemistry on cellular changes have been initiated. To evaluate the final fate of the altered tissues in both male and female systems, groups of mice and rats perinatally exposed to estrogens have been maintained to study development of neoplasms in target tissues and elsewhere. Some animals receive additional treatment of either estrogen or a chemical carcinogen at adult ages in an attempt to assess any changes in carcinogenic susceptibility of hormonally altered tissues during the perinatal period.

(2) To understand the mechanism of development of autoimmune oophoritis, studies on subcellular localization of antigen(s) in ooplasma and their nature have been started. Proposed projects include identification of the most essential participant among T-cell subpopulations in oophoritis and investigation of the most fundamental hormonal imbalances responsible for development of ovarian tumorigenesis.

Date Contract Initiated: March 1, 1975

Current Annual Level: $70,750

ALTON OCHSNER MEDICAL FOUNDATION (NO1-CP-65764)

Title: Study of the Influence of Repeated Low Dose Irradiation on Mammary Gland Carcinogenesis in Estrogenized Rats

Contractor's Project Director: Dr. Albert Segaloff

Project Officer (NCI): Dr. D. Jane Taylor

Objectives: To determine the carcinogenic effect of repeated, low dose irradiation on the mammary glands of estrogenized rats.

Major Findings: The contractor demonstrated previously that a single dose of x-ray delivered to one mammary chain of femal AxC inbred rats implanted with diethylstibestrol (DES) pellets resulted in a significant increase in mammary cancers over those resulting from the DES alone, the radiation alone or the sum of these. Cancers were also earlier in onset.

The optimal dose for this synergism was 150r to the left mammary chain. The initial protocol for this study was to duplicate the original experiment in order to compare animals given the 150r in a single dose with those getting 150r in 10r increments at four week intervals. When 25% DES pellets were employed none of the animals survived sufficiently long to have a significant survival period after the last of the 15 radiation treatments. Even though synergism was apparent in these animals their survival time after irradiation was not sufficient to assess optimal carcinogenesis. The controls given the 150r at the initiation of the experiment already had a large number of tumors and had started to expire before the split-dose animals had received half of their radiation dose.

The Brookhaven National Laboratory had demonstrated that when DES in the pellet was decreased to 8.3% there was a significantly longer latent period to the median cancer and the synergism was still apparent. Accordingly, an additional experiment was undertaken using the DES in this concentration and using 150r as the reference radiation dose. In this study the synergism is already apparent; however, the animals getting 10r every four weeks have just completed their radiation and those getting 5r every four weeks have only completed half of their projected radiation. In these latter groups synergism is not yet apparent.

Because animal mammary glands have relatively low amounts of fat and since obesity appears to be a factor in the development and prognosis of human breast cancer, obese rats have been produced by feeding them a highly palatable diet and restricting their activity. These obese rats have been implanted with 25% DES - 75% cholesterol pellets along with appropriate controls. They were given the optimal dose of 150r either in the standard fashion (perpendicular beam to the left mammary chain) or in such a way that the beam traverses the entire mammary chain from tail to head, thereby producing the gradient of x-ray exposure such as seen with mammography. This experiment has progressed to a point where a significant period of time has elapsed and tumors are beginning to appear. There is, however, at this stage only the suggestion that the synergism observed in previous studies is being observed and that there are more tumors at the entry of the radiation beam than at the exit.

Significance to Biomedical Research and the Program of the Institute: The question of possible carcinogenic effect of low dose radiation has not been adequately investigated because of the large number of animals required for significant findings. It is thought that the synergism with estrogen and radiation in the AxC rat presents an ideal means of testing low-dose radiation. This is particularly important because of the possible danger from repeated mammograms or other types of irradiation exposure, particularly in women who are obese and who are taking exogenous estrogens.

Proposed Course: Additional protocols have been developed for compressing the low-dose radiation into a shorter peiod of time and employing lower doses of radiation so that differences between single dose vs. repeated low doses may become more apparent. The preliminary study of the inter-

mittent administration of estradiol benzoate and a single dose of 150r of radiation has shown that there is at least a great prolongation in the latent period. Therefore, a comparison has been initiated employing estradiol delivered continuously from estradiol-cholesterol pellets.

Date Contract Initiated: September 30, 1976

Current Annual Level: $191,103

AMERICAN HEALTH FOUNDATION (N01-CP-75952)

Title: Studies of Colon Carcinogenesis in Organ Culture of Intestinal Mucosa

Contractor's Project Director: Dr. Gary M. Williams

Project Officer (NCI): Dr. Carl E. Smith

Objectives: To develop organ culture and transplant systems of mouse and rat colon in which to study mechanisms of chemical carcinogenesis and anticarcinogenesis.

Major Findings: Techniques have been developed for maintaining fragments of mouse and rat descending colon in organ culture for up to 28 days. During this interval, the mucosal epithelium remained viable and functional, and a relatively normal pattern of replication and turnover of mucosal epithelium took place.

Preliminary studies of the effects of chemical carcinogens on these organ cultures revealed that the colon carcinogens, methylazoxymethanol acetate, 1,2-dimethylhydrazine and azoxymethane inhibited replicative DNA synthesis in the mocosa and that N-methyl-N'-nitro-N-nitrosoguanidine induced DNA repair in explants in which replicative synthesis was suppressed by hydroxyurea.

Significance to Biomedical Research and the Program of the Institute: The availability of a colon organ culture system will permit refined mechanistic studies on the process of cancer causation in this organ which is one of the most frequent sites of human cancer.

Proposed Course: (1) The techniques for the maintenance of colon cultures will be further optimized; (2) The effect of long-term exposure of organ cultures to colon carcinogens will be studied with emphasis on the development of neoplastic lesions; and (3) Transplant techniques will be developed for assessing the development of neoplastic lesions in carcinogen-exposed cultures.

Date Contract Initiated: September 30, 1977

Current Annual Level: $94,336

AMERICAN HEALTH FOUNDATION (N01-CP-75948)

Title: Studies of Metabolic Capacity in Intestinal Mucosa

Contractor's Project Director: Dr. Emerich S. Fiala

Project Officer (NCI): Dr. Carl E. Smith

Objectives: 1. Determine, using germfree and conventional rats, the metabolic activity of the colonic microflora to metabolize a wide variety of precarcinogens, carcinogens and their metabolites, like 1,2-dimethylhydrazine, azoxymethane, methylazoxymethanol, 3,2'-dimethyl-4-aminobiphenyl and 3-methyl-2-naphthylamine and the metabolic capacity of the small intestinal and colonic mucosa to metabolize these compounds and their metabolites to the proximate and/or ultimate carcinogens.

2. To incubate radiolabeled compounds with colonic contents with small intestinal and colonic mucosal fractions and with isolated small intestinal and colonic loops from germfree and conventional rats and to identify the metabolic products formed.

3. To investigate the metabolic capacity of the liver and kidney in germfree and conventional rats and to metabolize the above precarcinogens and carcinogens to ultimate or proximate carcinogens and to compare such metabolism with that of the intestinal and colonic mucosa.

Major Findings: Preliminary findings in this new contract are:

1. Incubations of cecal contents of conventional F-344 rats with 1,2-dimethylhydrazine ($-^{14}CH_3$) indicate metabolism of this intestinal carcinogen to a compound tentatively identified as monomethylhydrazine. This metabolism does not occur with analogous preparations from germfree animals. Similarly, conversion of the colon carcinogens 3-methyl-2-naphthylamine and 3,2'-dimethyl-4-aminobiphenyl to as yet unidentified metabolites also appears to be occurring during incubation with cecal preparations from conventional but not germfree rats under anaerobic conditions.

2. The hydroxylation of the colon specific carcinogen azoxymethane to methylazoxymethanol in vitro by rat liver preparations is being studied in detail; a second metabolic product has been detected by high pressure liquid chromatography. Techniques for the isolation and identification of this second product of microsomal oxidation of azoxymethane are being developed.

3. Incubation of liver S-9 fractions with 3,2'-dimethyl-4-aminobiphenyl (^{3}H) results in the metabolism of the carcinogen and binding of label to the S-9 constituents. The degree of binding is decreased 50% by using liver S-9 fractions from disulfiram pretreated rats.

4. ^{14}C-labeled methylazoxymethanol acetate has been synthesized by a modification of the original Horisberger and Matsumoto procedure leading to a better yield and increased purity of product. The labeled carcinogen will be used in in vitro studies to examine possible metabolism by subcellular preparations from colon mucosa, liver and kidney.

Significance to Biomedical Research and the Program of the Institute: This program is designed to elucidate the mechanism whereby certain chemicals produce tumors specifically in the large bowel. Such organospecificity could derive from specific metabolic activation pathways present either exclusively or in greater levels in the colon mocusa as compared with other organs; an additional contributing factor may be the presence of bacteria in the gut which may further modify proximate carcinogens excreted into the bile. Several of the compounds being examined in this respect are similar to chemicals present in the environment and are especially relevant to the human condition in that analogous arylamine compounds (possessing a methyl group ortho to the amine) with extremely high mutagenic potential have been recently detected in pyrolyzates of meat and fish.

Proposed Courses: The in vitro incubation systems will be further refined so that the metabolic capacity of the small and large intestines mucosae may be compared with that of the liver and kidney as well as other organs. The products of both tissue and bacterial metabolism of the colon specific carcinogens will be identified and examined for mutagenicity and, where indicated, for carcinogenicity in rodents.

Date Contract Initiated: September 30, 1977

Current Annual Level: $100,879

AMERICAN HEALTH FOUNDATION (NO1-CP-75940)

Title: Long-Term Studies of Prevention of Epithelial Cancer by Retinoids

Contractor's Project Directors: Dr. John H. Weisburger
Dr. Jerald Silverman

Project Officers (NCI): Dr. Carl E. Smith
Dr. Michael B. Sporn
Dr. Jerrold M. Ward

Objectives: The objective of this program is to assess the effects of retinoids on epithelial tissue, specifically that found in the colon using the direct acting carcinogen N-methyl-N-nitrosourea and the indirect acting carcinogen 1,2, dimethylhydrazine to induce tumors. Since it is of major importance to be able to detect early neoplastic lesions of the colon, contractor will use endoscopy on the rats in this study to detect early colonic lesions. Contractor's long-term objective will be a dose

response study to determine what level of dietary retinoid is needed to prevent neoplastic changes.

Major Findings: This is a new contract and experiments have been under way since January, 1978. There are twenty-five groups of Fischer F344 rats involving a total of 667 animals given the colon carcinogens 1, 2, dimethylhydrazine subcutaneously, or N-methyl-N-nitrosourea intrarectally. There are control animals as well as rats given five distinct retinoid compounds.

Significance to Biomedical Research and the Program of the Institute: Supported by another Contract (NO1-CP-33208) American Health Foundation has pioneered in the development of animal models for colon cancer research. Several of these models are being applied to induce early colon tumors and to study whether the course of this induction process can be modified by retinoids. Collateral data will be collected to acquire information on the mechanism whereby any inhibition occurs. Colon cancer is the cancer with the highest incidence in the United States in particular, and in the Western world in general. Any valid information permitting a reduction of the incidence of this disease will be a valuable asset.

Proposed Course: 1) To continue the experiments designed to investigate the effect of retinoids on colon cancer induction in animal models; and 2) To acquire data bearing on the mechanism of this induction process.

Date Contract Initiated: September 30, 1977

Current Annual Level: $148,880 (Supported by Carcinogenesis Testing Program)

AMERICAN HEALTH FOUNDATION (NO1-CP-33208)

Title: Studies in Colon Carcinogenesis

Contractor's Project Director: Dr. John H. Weisburger

Project Officer (NCI): Dr. Carl E. Smith

Objectives: Colon cancer is the neoplastic disease with the highest incidence in the United States. Epidemiological studies indicate that the occurrence of colon cancer is associated with a high dietary intake of fat and beef and with a lack of dietary fiber. The overall aim of this program is to acquire an understanding of the underlying multifactorial complex causative mechanisms and eventually develop a sound, rational basis for preventive measures. Animal models and bioassay systems are developed and utilized to uncover a relationship between colon cancer and total dietary fat, hidden fat as present in meat and fiber. The role of the intestinal bacterial flora is assessed, since this element participates in the overall metabolism of carcinogens, cocarcinogens, and dietary elements as well as endogenous digestive secretions. Key substrates of

interest are acid and neutral sterols in relation to macro and micro constituents of the diet. Modification of colon carcinogenesis through exogenous and endogenous chemicals, food additives, and diet components in general is under investigation in conventional and germ-free animal models and in man.

Major Findings: The carcinogen 3,2'-dimethyl-4-aminobiphenyl (DMAB) induced a lower yield of colon tumors in male and female germ-free F344 rats and fewer breast tumors in male in female germ-free female rats compared with conventional rats. This suggests that the intestinal microflora play a modifying role, directly or indirectly, in DMAB-induced carcinogenesis. The mechanism requires definition.

The primary bile acids, cholic acid and chenodeoxycholic acid, acted as colon tumor promoters in conventional F344 rats but not in germ-free animals. This was probably due to production of deoxycholic acid and lithocholic acid by the bacterial 7α-dehydroxylation of cholic acid and chenodeoxycholic acid in the colon of conventional rats extending and confirming contractor's previous findings that the secondary bile acids, deoxycholic acid and lithocholic acid, promoted colon cancer induction in germ-free rats. Neutral sterols, such as cholesterol, cholesterol epoxide and cholestan -3β-5α, 6β-triol did not act as colon tumor promoters in germ-free or in conventional F344 rats. This definitive null effect is of importance in relation to mechanism proposed in the past.

In conventional F344 rats, bacterial nitrosation of a secondary amide does not occur in the colon in sufficient amounts to elicit colon carcinogenesis.

In relation to diet and colon carcinogenesis, a high-fat (20%) compared to a low-fat (5%) diet increased the colon tumor incidence induced by 1, 2-dimethylhydrazine, methylazoxymethanol acetate, or methylnitrosourea in conventional F344 rats. These results indicate that irrespective of the type and mode of action of colon carcinogens used, direct acting or requiring metabolic action, the animals fed a high-fat diet had a higher colon tumor incidence than did those fed a low-fat diet.

Experiments are continuing to study the effect of lifetime intake of diets containing high animal and vegetable protein and fat to two generations of rats with respect to spontaneous tumor incidence with emphasis on large bowel. Additional tests involve the determination of neutral and acid sterols in the stools of these animals. Additional studies have extended the preliminary report that the tumor yield is higher in rats given methylnitrosourea with a surgical anastomosis in the colon. The mechanism of this effect is under study.

It has been demonstrated in animal models that bile acids have a promoting effect in colorectal carcinogenesis. The effect was seen in animals receiving the bile acid under study in the presence of the normal luminal bile flow. A series of studies is continuing in which all animals at risk were given the direct acting carcinogen methylnitrosourea intrarectally.

After 10 and 24 weeks respectively a colostomy was performed, thus removing bile flow and luminal content flow generally from a portion of the gut pretreated with carcinogen. Rats in which the colostomy was performed after 10 weeks, and therefore where the bile flow was diverted over a 22-week period, had no colon cancer whereas over 80% of controls had colon cancer. In the second series of experiments now underway, the carcinogen methylazoxymethanol was given intrarectally or intraperitoneally to rats with a colostomy established one week previously. Deoxycholic acid was infused intrarectally repeatedly into the remaining stump in order to determine whether this bile acid by itself promotes colon cancer in the absence of normal bile flow.

In relation to fiber and colon cancer, a comparison was made between a high-risk population from New York and low-risk population from rural Kuopio, Finland. In spite of equivalent high dietary fat intake and hence total daily fecal bile acids, one of the factors contributing to the low risk of colon cancer in Kuopio is the fact that a high dietary fiber, mainly from bread cereals, leads to an increase in fecal bulk. Thus, promoters such as bile acids and yet to be identified carcinogens are diluted. This is the first definite evidence that natural fiber plays an inhibiting role in colon cancer in man and provides leads to the underlying mechanisms.

Along these lines, model studies in F344 rats showed that diets containing 15% wheat bran or pectin, but not alfalfa, inhibited azoxymethane-induced colon tumor incidence. On the other hand, diets containing bran or pectin had no protective effect in methylnitrosourea-induced colon carcinogenesis, but the animals fed alfalfa diet had a higher colon cancer incidence than those fed a control diet. Thus, the protective effect of fiber in colon carcinogenesis depends on the type of carcinogen and the sources of fiber. The data also reveal that 15% undegraded carrageenan (Viscarin 402) in the diet had a promoting effect in colorectal carcinogenesis evoked by azoxymethane or methylnitrosourea.

High pressure liquid chromatographic methods were developed for the analysis of benzo(a)pyrene (BaP) in charcoal broiled meat and in rat and in human feces. Broiled hamburgers contained 52.7 ± 2.0 μg/kg BaP. The broiled meat was fed to male F344 rats and the feces analyzed for BaP. The unchanged BaP in the feces amounted to 11.3% of the BaP in the meat consumed. This value agrees well with model studies in which rats were dosed with labeled BaP. To determine the excretion of BaP in humans, eight male volunteers consumed charcoal broiled meat and then feces were collected. One week later the volunteers consumed an equivalent quantity of meat that had been cooked in aluminum foil which prevents contamination with BaP. The analysis of these samples is currently in progress.

Significance to Biomedical Research and the Program of the Institute: The program is of special significance since it is designed to provide important information on the relationship between diet, intestinal microflora, and colon carcinogenesis in realistic animal models. In parallel, supported by other funds (USPHS-NCI Grant CA-16382 from the National Large

Bowel Cancer Project), research on the relationship of intestinal microflora, acid and neutral sterols, and other key metabolites in varied human population groups on distinct nutritional regimens is underway. It is hoped that the data base thus generated in animal models and in man can significantly enhance our knowledge of the controllable etiological factors which play a role in cancer of the large bowel. The recent finding that dietary fiber can inhibit development of colon cancer is an important, logical development along these lines. The long-term goal is to provide a basis for rational prevention of disease affecting over 100,000 individuals per year in the United States.

Proposed Course: (1) To investigate the effect of high and low-risk diets on carcinogenesis in germ-free and conventional animals utilizing MNU, DMAB and DMH as carcinogens; (2) To determine the effect of relevant exogenous chemicals and food additives in these systems; (3) To assess further the promoting and cocarcinogenic activity of endogenous and exogenous chemicals in colon bioassay system; (4) To study the detection of carcinogens in excreta and tissues through application of sensitive and specific analytic and rapid bioassay methodology; (5) To study the protective effect of micronutrients, such as Vitamins C and E, and selenium in colon carcinogenesis; and (6) To study the mechanism whereby bile acids act as promoters of carcinogenesis in the large bowel but not on mouse skin.

Date Contract Initiated: June 30, 1971

Current Annual Level: 0

AMERICAN TYPE CULTURE COLLECTION: (N01-CP-65751)

Title: Development of Cell Strains from Ductal and Exocrine Portions of the Pancreas

Contractor's Project Director: Dr. Robert J. Hay

Project Officer (NCI): Dr. Stuart Yuspa

Objectives: To establish reproducible techniques for the isolation and maintenance or cultivation of pancreatic duct and exocrine cells. Such cultures will then be used for studies on chemical carcinogensis.

Major Findings: Conditioned media from 14 established lines were tested previously for their ability to stimulate proliferation of pancreatic epithelia from strain 13 guinea pigs. Of the seven found to be active, media from human lung fibroblast strains WI-38 and MRC 5 were most consistently effective, providing cell yields 40 to 105% higher than controls after 7 days. Since the effect was transitory, experiments were conducted in which x-irradiated lung fibroblasts were used as feeder layers for acinar epithelia. Marked stimulation was observed in morphological and

biochemical studies. Epithelial cells in the presence of feeder layer continued to proliferate even after 7 weeks in culture, while controls degenerated after 20 days. When examined by pulse labeling with H-thymidine followed by extraction and quantitation, acinar cell cultures with feeder layer incorporated more than twice as much isotope than did controls. Verification was accomplished by autoradiographic analyses. While more than 40% of the acinar cell nuclei were labeled in 8-day cultures containing irradiated fibroblasts, only about 9% were labeled in controls. These results suggest that cellular interactions may be required for long-term maintenance of acinar epithelia in culture.

Epithelia from pancreatic ducts of Syrian hamsters have been maintained in explant culture for over 6 weeks. Cytochemical markers were utilized to demonstrate similarity between the epithelia in culture versus those lining the pancreatic duct in situ. Ductal epithelia from quinea pig pancreatic tissue have been isolated by dissociation of the luminal layer. The cells can be retained in culture as colonial aggregates in a manner similar to that described previously for acinar epithelia.

Significance to Biomedical Research and the Program of the Institute: The development of model systems for the cultivation of specific pancreatic duct and exocrine cells would provide powerful tools for studies of carcinogenesis. Definition of the factors that promote continued proliferation and function in such systems would also contribute to the basic understanding of pancreatic epithelial cell physiology.

Proposed Course: Further definition of the requirements for subcultivation and long-term propagation of pancreatic epithelia is need. The role of the feeder layer is of obvious interest as is the possible effect of humoral agents known to affect pancreatic growth and function. Parallel culture effort with human tissue is planned.

Date Contract Initiated: February 1, 1976

Current Annual Level: $88,851

BAYLOR COLLEGE OF MEDICINE: (NO1-CP-65837)

Title: Markers for Evaluation of Preneoplastic Lesions in the Respiratory Tract.

Contractor's Project Directors: Dr. S. Donald Greenberg
Dr. Harlan J. Spjut
Dr. Stephen L. Kimzey

Project Officers (NCI): Dr. Michael B. Sporn
Dr. Carl E. Smith

Objectives: The objective of this task is to identify specific morphometric features of cells from the human respiratory tract which are indicative of a very early stage in the development of neoplasia. Optical data digitizing techniques and computerized image analysis procedures are employed as these techniques produce both highly repeatable and objective results.

Major Findings: 1. Stages of squamous cell carcinogenesis can be recognized using computerized image analysis and are amenable to description by statistically evaluated properties. 2. Color changes in the cells associated with different stages of carcinogenesis can be quantified and used in an overall description of this process. 3. Preliminary results suggest a statistically significant difference between metaplastic cells of subjects who develop dysplasia and those who do not. More data are needed to substantiate this finding.

Significance to Biomedical Research and the Program of the Institute: In excess of 80,000 deaths a year, with an associated economic loss of $2 billion per year, are attributed to lung cancer. Diagnostically, lung cancer often remains asymptomatic until late in its development, a development which may span 10-40 years. With these biological characteristics, a reasonable approach to control this disease is prevention.

However, the major causative agent for lung cancer, inhaled tobacco smoke, is related to social behavior patterns which are not easily controlled. As control is difficult, early diagnosis becomes important. Contractor's project objective is to identify those individuals with a significant risk of developing lung cancer and to do this considerably in advance of the development of malignant disease and at a stage where the disease process is easily reversible. This corresponds to the Institute's objective of improving diagnostic procedures for early detection and possible prevention of the disease.

Proposed Course: 1. A high-speed optical data digitizing system will be completed in June, 1978. This system will be used to expand rapidly an existing data base of normal and abnormal lung cells. 2. Metaplastic cells show differences which appear to correlate with the presence or absence of dysplasia in a subject. Data in these areas will be augmented and examined extensively to substantiate or refute this finding. 3. Other cells found in the lungs will be examined to determine if their morphometric characteristics correlate with the progress of squamous cell carcinogensis. 4. Color analysis of cells will be explored in more detail. A description of squamous cell carcinogenesis will be generated based on color information. The limitations of these methods will be examined.

Date Contract Initiated: September 30, 1976

Current Annual Level: $85,400

BIOTECH RESEARCH LABORATORIES, INC. (N01-CP-55640)

Title: Criteria for In Vitro Cell Transformation by Viruses and Chemical Carcinogens

Contractor's Project Director: Dr. Robert C.Y. Ting

Project Officer: Dr. Stuart H. Yuspa

Objective: To establish criteria for in vitro carcinogenesis which will serve as in vitro endpoints that correlate with tumorigenic potential.

Major Findings: Studies utilizing fibroblastic and epithelial cell lines from a variety of species have demonstrated unequivocally that a cell aggregation assay developed during this program is a very reliable indicator of in vitro cell transformation. These investigations indicate that cell survival in the aggregate form correlates very well with colony formation in soft agar and tumorigenicity in nude mice and/or syngeneic hosts. In the aggregate form, normal cell populations display a rapid decline in the number of viable cells over a 4-5 day period, whereas counterpart transformed cell populations in the aggregate form exhibit an ability to survive and, in many cases, to proliferate. This aggregation system can also be used in a selective manner for eliminating normal cells from a cell population and rapidly increasing the number of transformed cells in the population.

Significance to Biomedical Research and the Program of the Institute: In vitro indices of cell transformation that can successfully predict tumorigenicity would be of great value in screening programs for potential carcinogens. Such criteria would also provide a means of monitoring in vitro transformation of human cells.

Proposed Course: Contract terminated October 15, 1977

Date Contract Initiated: October 15, 1974

Current Annual Level: 0

CALIFORNIA, UNIVERSITY OF (N01-CP-75934)

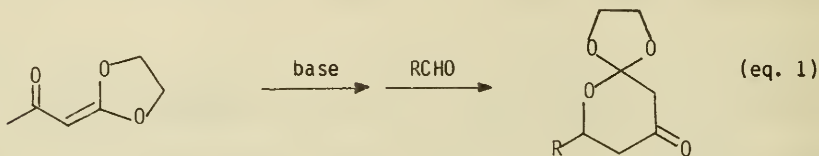
Title: Synthesis of New Retinoids for In Vitro Studies of Lung Cancer and Other Epithelial Cancers

Contractor's Project Director: Dr. Clayton H. Heathcock

Project Officers (NCI): Dr. Carl E. Smith
Dr. Michael B. Sporn

Objectives: Synthesis of retinoids for in vitro studies

Major Findings: Contractor has developed a general synthesis of compounds of the type 1 (see equation 1 below), and is applying this synthesis to the preparation of analog 2. Compound 2 is important since it is closely related to 3, which is so far the compound of greatest therapeutic index in the retinoid program.



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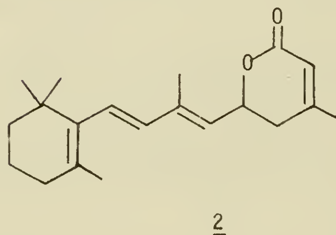

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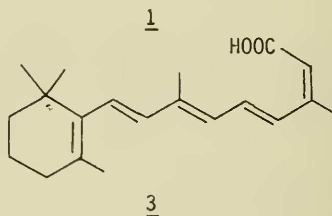

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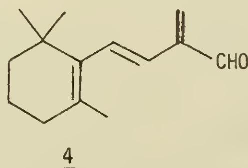
Contractor has also synthesized the simple model aldehyde 4, and is extending this aspect of the project to compound 5, an attractive candidate for activity in the retinoid project.



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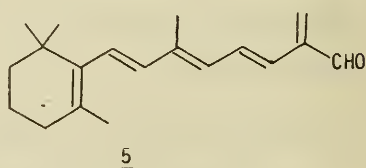

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Significance to Biomedical Research and the Program of the Institute: Retinoids (the set of molecules comprised of vitamin A and its synthetic analogues) are known to exert profound effects in controlling cellular diferentiation in epithelia of many target tissues. Large numbers of synthetic retinoids have been made, in which there has been substantial modification of the ring, side chain, or polar terminal group of the natural vitamin A molecule. Recently, it has been shown that several

synthetic retinoids can prevent the development of epithelial cancer of the skin, respiratory tract, mammary glands, and bladder in experimental animals. Whether or not this will turn out to be a practical approach to the problem in man is not yet known. Such determination of the potential these compounds have for specific human epithelial cancers is of the highest significance and requires a variety of in-depth investigations.

Proposed Course: Retinoids will continue to be synthesized at the direction of the Project Officer for testing of efficacy in anticarcinogenesis studies of the NCI.

Date Contract Initiated: September 30, 1977

Current Annual Level: $51,054

CHICAGO, UNIVERSITY OF (N01-CP-65777)

Title: Markers for Evaluation of Preneoplastic Lesions in the Respiratory Tract

Contractor's Project Director: Dr. George L. Wied

Project Officer (NCI): Dr. Michael B. Sporn

Objectives: The major objective is to describe markers which differentiate between the reversible and irreversible changes in a cell population during its development toward frank cancer.

Major Findings: Two different experiments have been done to establish preneoplastic changes in squamous epithelial cells in the respiratory tract.

In the first experiment a group of hamsters were exposed to Benz[a]Pyrene by intratracheal instillation of the carcinogen. A control group of animals treated by ferric-oxide or an alcohol wash, respectively, were taken at varying time intervals, but only those from control animals and from the animals with squamous cell cancer were scanned by three color photomultiplier technique of the TICAS system. The cells were evaluated by cytopathologists and cytotechnologists as to their degree of atypia. Digitized cell images were then analyzed by computer using the best 25 cell descriptive features, selected by number of multivariate statistical methods from more than 200 features in total. Classification results of supervised and unsupervised (cluster) pattern recognition analysis of cell images were then compared with those of a cytopathologist. Postmortem examination was done on all hamsters and comparison of the morphology of the tumor cytology and histology was accomplished.

The second experiment using N-Methyl N-Nitroso Urea instead of Benz[a]-Pyrene is now in progress. The treatment and analytical techniques used here are similar to those used in the first experiment. Preliminary results have been obtained.

The following is a brief description of results obtained by mathematical analysis of recent data from the lung project.

The number of digitized 3-color images of well-stained and preserved cells obtained during the last 8 months more than doubled contractor's lung cells data bank. The presently available pool consists of more than 3000 cell images.

From selected cells the six basic cell groups were formed for mathematical analysis. These include two control groups (one from animals without treatment and one from animals treated with ferric-oxide) and four additional cell groups from animals treated by the carcinogen benz[a]pyrene which are defined according to their degree of abnormality (two groups with metaplastic changes, a third group with high nuclear-cytoplasmic ratio and possibly preneoplastic changes and a fourth group with preneoplastic changes and recognized attributes of a cancer cell).

The best 25 pattern recognition features were used in a number of multivariate tests to evaluate the kind and degree of similarities of the analyzed cell types. These features are defined by color, shape, texture, and many other morphologic parameters. The obtained results demonstrate the capability of the TICAS system to recognize and separate the control cell groups from preneoplastic and from cancer cells in the pulmonary aspirates of hamsters.

Significance to Biomedical Research and the Program of the Institute: Determination of feasibility to assess reversible and irreversible cell changes by means of an objective, automated pattern recognition system.

Proposed Course: The development of improved techniques to differentiate between reversible and irreversible changes in respiratory epithelium by use of TICAS analysis.

Date Contract Initiated: September 30, 1976

Current Annual Level: $141,900

CHILDRENS HOSPITAL OF LOS ANGELES (NO1-CP-55641)

Title: Fibrinolysis as a Parameter of In Vitro Transformation

Contractor's Project Director: Dr. William F. Benedict

Project Officer (NCI): Dr. Stuart H. Yuspa

Objective: The main objective of this contract is to determine association of increased plasminogen activator production with malignant transformation and to determine whether an increased production of this protease is an early marker of transformation. In addition, the subcellular localization of plasminogen activator is being sought as well as whether

increased plasminogen activator is an expression of invasive potential of certain cells.

Major Findings: (1) In collaboration with Dr. Nancy Colburn at the NIH, it has been demonstrated that transformed mouse epidermal cells produce high levels of plasminogen activator. The increase of this protease during malignant transformation seems to appear concurrently with the ability to grow in soft agar but before the capability to grow as tumors in immunosuppressed animals. (2) It has been found that endothelial cells derived from the aorta of newborn calves have high fibrinolytic activity. These normal cells, which can invade tissue upon an appropriate stimulus, grow in soft agar and in the presence of a specific serum show morphological changes usually observed in transformed cells. They remain diploid, however, and do not grow as tumors in the nude (athymic) mice. The plasminogen activator secreted by these endothelial cells has been isolated and purified. (3) The cell cycle specificity of plasminogen activator production in malignant cells has been shown to be different than normal endothelial cells. (4) It has been found in collaboration with Dr. Marco Baggiolini that plasminogen activator in the human fibrosarcoma cell line HT-1080 is localized either in special segments of the plasma membrane or in small vesicles. Plasminogen activator secreting and nonsecreting clones showed an identical subcellular distribution pattern. The plasminogen activator secreted by the HT-1080 cells has been isolated, purified and characterized. Antisera has been obtained from rabbits immunized with this purified protease.

Significance to Biomedical Research and the Program of the Institute: The association of increased production of plasminogen activator with malignant transformation has been well documented by contractor as well as others and is again underlined by the presented results. These findings also indicate that an increased production of this protease may be an early marker of transformation. Furthermore, it appears that increased plasminogen activator production may be an expression of invasive potential of normal and transformed cells and that the cell cycle production of this protease differs in normal and transformed cells.

Proposed Course: (1) Study the relationship of increased plasminogen activator production to transformation in human cells; (2) Investigate the subcellular localization of this protease in different normal (endothelial cells and transformed cells, including the use of immunofluorescence techniques; and (3) Continue to study the mechanisms of induction and inhibition of plasminogen activator production in normal and transformed cells with invasive potential.

Date Contract Initiated: October 21, 1974

Current Annual Level: $68,943

COLORADO, UNIVERSITY OF (NO1-CP-75875)

Title: Tissue Interactions in Induction and Perpetuation of Hormonally-Induced Permanent Cellular Alterations

Contractor's Project Director: Dr. Gerald R. Cunha

Project Officer (NCI): Dr. Jerry M. Rice

Objectives: To determine the role of tissue interactions in normal, abnormal, and neoplastic growth and differentiation of the vagina, paying particular attention to the role of stroma in expression of epithelial differentiation; and to investigate the mechanism of cell-cell interactions in normal and abnormal development of the urogenital tract.

Major Findings: Development of the uterus, vagina, cervix, and prostate requires an interaction between urogenital epithelium and its subadjacent mesenchyme which specifies the morphogenetic pattern of the epithelium. Similarly, development and expression of estrogen-induced irreversible effects in the developing vagina (ovary independent persistent vaginal cornification) requires an interaction between epithelium and stroma in which the stroma induces vaginal epithelial hyperplasia. The mechanism of these interactions may involve the transfer of macromolecules, because it appears that basal vaginal epithelial cells transport basal laminar material in a directionally specific manner which corresponds to the known direction of inductive affects; epithelial cells endocytose and exocytose this material.

Ultrastructural data corroborates the findings of other investigators that induction of alterations in vaginal cytodifferentiation by neonatal exposure to estradiol may occur through a mechanism of vaginal epithelial cell selection. Consistent with this theory is the finding in this contract that in estrogen-treated vaginae, hemidesmosomes (putative attachment organelles between basal epithelial cell and the underlining basal lamina) are approximately 4 times as numerous in the basal vaginal epithelial cells which apparently displace neighboring cells from the basal lamina.

Although stromal effects on epithelial morphogenesis are well established, it is uncertain whether stroma has an effect upon expression of biochemical markers in urogenital epithelium. In tissue recombinants between a prostatic inductor (urogenital sinus mesenchyme) and bladder or skin epithelia, the heterotypic epithelia undergo prostatic morphogenesis and demonstrate a new set of histochemical characteristics indicative of prostatic epithelium.

Significance to Biomedical Research and the Program of the Institute: The normal morphogenesis and cytodifferentiation of many embryonic structures has been shown to be dependent on an interaction between epithelium and mesenchyme. In addition, maintenance of adult structure and function also appears dependent on such an interaction, and some investigators have

suggested that carcinogenesis may occur as a result of an alteration of the normal interaction between epithelium and stroma. This hypothesis, for technical reasons, has not been confirmed. However, use of the vagina as a model system provides a technically-feasible approach to this fundamental question.

In addition, the research deals with the broader question of how intercellular communication occurs in various morphogenetic tissue interactions in embryonic, neonatal, and adult organs. It is believed that understanding of normal processes of morphogenesis and cytodifferentiation will provide the necessary background to permit formulation of protocols which will directly determine the relationship between perturbations of the normal process of cytodifferentiation and the development of carcinoma.

Proposed Course: Recombinations of epithelium and stroma from vaginas of estrogenized and untreated female mice are being repeated, and the grafts allowed to grow in ovariectomized hosts for 12 to 18 months to determine if stromal induction of epithelial hyperplasia and parakeratosis will lead to epidermoid carcinoma of the untreated vaginal epithelium. In addition, an extensive series of heterotypic recombinations between epithelium and stroma from various regions of the reproductive, integumentary, and digestive systems are being analyzed in order to determine the roles of epithelium and stroma in the normal pre- and postnatal development of the reproductive tract.

Studies on the mechanism of vaginal epithelial cell seletion via differential cellular adhesion (hemidesmosomes) and the mechanism of tissue interactions via epithelial uptake of the extracellular matrix will continue through employment of untrastructural, autoradiographic, histochemical and immuno-cyto-chemical approaches, coupled with contractor's trypticseparation and recombination technique.

Date Contract Initiated: December 15, 1976

Current Annual Level: $145,051

COLORADO, UNIVERSITY OF (N01-CP-65849)

Title: In Vitro Cultivation of Normal Epithelial, Human Prostatic Cells

Contractor's Project Director: Dr. Mukta M. Webber

Project Officer (NCI): Dr. Stuart H. Yuspa

Objectives: The major objective of this NCI program is to develop an in vitro model which could be used to investigate early steps in human prostatic carcinogenesis. In order to develop this model, procedures must be developed for the cultivation of prostatic epithelium of normal human origin in pure cultures. The immediate objectives are to isolate normal human prostatic epithelium; to establish nutrient requirements for its

growth and maintenance in vitro; and to characterize the epithelium grown in vitro to establish its normal, human prostatic origin.

Major Findings: Major emphasis has been placed on 1) Establishing nutrient requirements of normal human prostatic epithelium; 2) Quantitation of results from experiments conducted to establish optimum levels of these nutrients; and 3) Characterization of cells isolated and grown in vitro.

A method for separation of prostatic acini from stroma using collagenase was established earlier in this study. This method has been used to establish vigorously growing cultures of prostatic epithelium. Such cultures have been used to evaluate the effects of nutrients which stimulate the growth and increase the lifespan of human prostatic epithelium in vitro. Only qualitative results were reported in the last report. Results from 30 sets of experiments and approximately 6000 cultures have now been quantitated and statistically analyzed. On the basis of these results, the type and optimum level of the following factors has been established for the growth of normal human prostatic epithelium in vitro: 1) type of medium; 2) type of serum; 3) concentration of serum; 4) concentration of glutamine; 5) concentration of insulin; and 6) concentration of 5 α-dihydrotestosterone.

A new method for quanitative analysis of growth using a Datacolor Scanning Densitometer has been developed. This method is reliable and very economical on time, hence has proven very useful in a project requiring the evaluation of several thousand cultures. In addition, measurement of total protein per culture and incorporation of ^{3}H-thymidine for evaluation of the effects of nutrients on growth are also being used.

Cells grown in vitro are being characterized by three methods: 1) Characteristics of cells at the ultrastructural level; 2) A cytochemical method for identifying prostatic acid phosphatase; and 3) Indirect immunofluorescence using antiprostatic acid phosphatase antiserum.

Significance to Biomedical Research and the Program of the Institute: The primary goal of the studies under this program is to determine the early biochemical and cellular changes which are involved in human prostatic carcinogenesis. Before these studies can be conducted, it is essential to have a model system. The specific objective of this study, therefore, is to develop an in vitro model which could be used to investigate early steps in prostatic carcinogenesis. Such a model system might have significance not only for studies on the early steps in carcinogenesis, but also for 1) target cell-carcinogen interaction; 2) the role of multiple agents and co-carcinogens in transformation; 3) testing chemotherapeutic agents; 4) as antigenic targets for monitoring cell-mediated anti-tumor activity in studies on immunotherapy; 5) as a cell system for viral vaccine production; 6) screening drugs, food additives and evironmental carcinogens and for forecasting their effects; 7) studies on cell nutrition, metabolism, growth, differentiation and aging; and 8) for studies on the role of cell aging in prostatic carcinogenesis.

Proposed Course: Studies on growth enhancement and maintenance will be continued using male sex hormones and their metabolites and vitamins. Various nutrients will be tested in combination, and finally a culture medium containing optimum levels of various nutrients essential for prostatic epithelial cell growth and maintenance in vitro will be developed.

Date Contract Initiated: September 30, 1976

Current Annual Level: $140,400

COLUMBIA UNIVERSITY (N01-CP-75897)

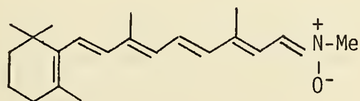
Title: Synthesis of New Retinoids for In Vitro Studies of Prevention of Lung Cancer and other Epithelial Cancers

Contractor's Project Director: Dr. Koji Nakanishi

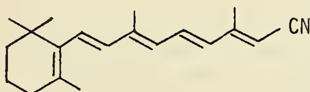
Project Officers (NCI): Dr. Carl E. Smith
Dr. Michael B. Sporn

Objectives: To synthesize retinoids which lead to regression of precancerous tissues.

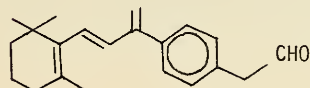
Major Findings: During the short time that this contract has been in effect, a number of retinal structures have been synthesized and their properties assessed. Methylated retinals and dihydroretinals have been found to be very unstable, unlike the dihydroretinoic acids. (The entire series of dihydroretinals has been made and found unstable.) Efforts are currently being made to synthesize the following retinoids in the near future:



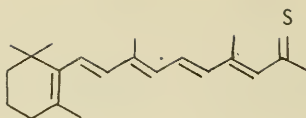
retinyl nitrone



cyano retinal (terminal nitrile)



aromatic retinal



thio-retinone
(terminal thio-methyl ketone)

Significance to Biomedical Research and the Program of the Institute: The structure-activity relation of the retinoids are still far from clear. Unusual retinoids may provide new types of structure which have satisfactory ratios of anticarcinogenic potency to toxicity as well as desirable pharmocokinetic properties.

Proposed Course: Retinoids will continue to be synthesized at the direction of the Project Officer for testing of efficacy in anticarcinogenesis studies of the NCI.

Date Contract Initiated: September 30, 1977

Current Annual Level: $78,835

DARTMOUTH COLLEGE (N01-CP-65776)

Title: Development of Animal Model for Oat Cell Carcinoma of the Lung.

Contractor's Project Director: Dr. George D. Sorenson

Project Officer (NCI): Dr. Michael B. Sporn

Objectives: To develop a satisfactory animal model of oat cell carcinoma of the lung either through xenogenic transplantation of human oat cell carcinoma cells from established cell lines or through primary induction of an analogous type of animal tumor.

Major Findings: One approach toward establishing an animal model system for study of small cell anaplastic carcinoma of the lung is being carried out utilizing congenitally athymic nude (nu/nu) mice inoculated with continuous cell lines isolated from primary metastatic tumors obtained from patients with a known diagnosis of this disease. The advantage of this model is that the tumor cells are of human origin. The cell lines synthesize and secrete various peptide hormones as well as estradiol.

The effect of antilymphocyte serum (ALS) and antithymocyte serum (ATS) on tumor growth from 5 cell lines was evaluated in both male and female mice. Percent tumors arising after inoculation subcutaneously of 10 cells was found to be significantly higher in untreated males than in untreated females, but no sex difference was observed when mice were treated with either ALS or ATS. One cell line produced no tumors.

In those mice with tumors, 3 characteristics of tumor growth have been evaluated: latent period before tumors reached 0.1 cm^3, initial doubling time and the subsequent rate of decline in growth. Few statistically significant differences were observed in mice inoculated with the same tumor cells; differences between tumors from different cell lines are being evaluated.

A comparison of growth of DMS 79 cells (high ACTH producer) in vivo and in vitro, by cell cycle analysis, showed essentially the same theoretical growth rate in both situations. However, cell loss due to death in tumors in vivo was high, resulting in an observed doubling time of 11.1 days, compared to 1.9 days in vitro.

An approach toward establishing an animal model system for the study of small cell anaplastic carcinoma induction in the lung is being carried out with hamsters. Hamsters are being inoculated weekly, subcutaneously with diethylnitrosamine. Groups of animals are being sacrificed biweekly for examination by routine light and electron microscopy as well as by fluorescent microscopy to evaluate autofluorescence of Kulchitsky cells. At this time animals have been killed up to 8 weeks of treatment. Atypical changes are observed in the epithelial cells lining the respiratory passages and there are apparently a few hyperplastic foci of Kulchitsky cells.

Significance to Biomedical Research and the Program of the Institute: To understand the pathogenesis of this cancer and to provide a model in which biological and other characteristics and responses of "oat" cells can be examined.

Proposed Course: Current studies are underway to evaluate transplantability of human cell tumors in nude athymic mice. A selection of the best system for future use in evaluating factors which may affect growth of tumor cells in vivo and in vitro will be made.

Hamsters treated with DEN will be studied over an additional 2-month period with the expectation that neoplasms will develop.

Date Contract Initiated: September 30, 1976

Current Annual Level: $157,810

DARTMOUTH COLLEGE (NO1-CP-55708)

Title: Chemical and Structural Requirements for Pancreas Uptake and Excretion: Rats and Hamsters

Contractor's Project Director: Dr. Thomas J. Curphey

Project Officer (NCI): Dr. Carl E. Smith

Objectives: To determine the chemical and structural properties of compounds having a propensity for pancreas uptake and excretion through the pancreatic duct.

Major Findings: Radiochemical tracer techniques have been used in studies of distribution of sixty-eight compounds into plasma, pancreas, liver, and kidney of rats and hamsters. Evidence for excretion into the pancreatic duct system has been sought. Active uptake by the pancreas has been found for a variety of alpha amino acids. The pancreas appears to show a low degree of structural selectivity in this uptake of alpha amino acids, provided both free amino and carboxyl groups are present. Acylation or dialkylation of the amino group or esterification of the carboxyl group abolishes active uptake, while monoalkylation on nitrogen or on the alpha carbon atom has little effect. Preliminary data suggest that optical configuration at the alpha carbon atom may not be a significant structural requirement for uptake of radioactive alanine, both D and L isomers being taken up to a similar extent. Studies with labeled polycyclic aromatic hydrocarbons have shown an initial rapid uptake of radioactivity by the pancreas, followed by a slower wash out. Of other compounds known or suspected to be pancreatic carcinogens (NMU, AAF, DAB, azaserine, benzidine, and BHP) only azaserine appears to have a marked affinity for the pancreas. However, animals receiving NMU excrete considerable radioactivity into the pancreatic duct system. The nature of this activity is not known.

Significance to Biomedical Research and Program of the Institute: The goal of this project is to define the structural determinants for localization of chemicals in the pancreas as a result of absorption and distribution through the blood stream, and further to determine which types of compounds might be excreted into the duct system. Such knowledge is sought to form a basis for understanding which, if any, environmental chemical carcinogens are to be most suspect as possible causes of carcinoma of the pancreas. Identification of such chemicals would form a basis for decreasing exposure and risk in humans. A further benefit of defining the structural determinants for pancreatic localization is that it would permit rational design of pancreas-specific carcinogens (for model studies), chemotherapeutic agents, and organ visualizing agents.

Proposed Course: Final studies will be made of localization of amino acids and related derivatives, known pancreatic carcinogens, and selected polycyclic aromatic and heteroaromatic compounds.

Date Contract Initiated: June 30, 1975

Current Annual Level: $0

DARTMOUTH COLLEGE (N01-CP-33378)

Title: Enhanced Delivery of Synthetic Nitroso Compounds to the Pancreas in Rats

Contractor's Project Director: Dr. Daniel S. Longnecker

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The principal goal is to establish an animal model for induction of pancreatic adenocarcinoma in rats using chemicals with two characteristics: 1) compounds which concentrate in the normal pancreas; 2) compounds which appear to be potential carcinogens on the basis of chemical structure, mutagenic activity in bacteria, and/or ability to cause malignant transformation of mammalian cells in culture. To achieve these characteristics seveal new nitroso derivatives of amino acids have been synthesized.

Major Findings: Testing of several compounds for carcinogenic effects in the pancreas of rats in long-term studies has continued. Three nitrosourea amino acids, N^{ε}-(N-methyl-N-nitrosocarbamoyl)-L-lysine (MNCL), N^{δ}-(N-methyl-N-nitrosocarbamoyl)-L-ornithine (MNCO), and N^{γ}-(N-methyl-N-nitrosocarbamoyl)-L-2,4-diaminobutyric acid (MNDABA), were studied for toxic and carcinogenic effects in rats. Acute cytotoxicity of the latter 2 compounds for pancreas was studied histologically and by electron microscopy. Single IP injections of 1-3 mmole/kg caused focal necrosis of pancreatic acinar cells reaching peaks of severity in 48-72 hours followed by regeneration. In 6-month studies all three compounds caused a high incidence of atypical acinar cell nodules after 6 weekly injections. Two nodules of highly dysplastic cells from animals treated with MNDABA were classed as carcinoma-in-situ and seemed to be of acinar cell origin. All three compounds were more effective as carcinogens in breast, skin and kidney than in pancreas. In a 1-year study of the carcinogenicity of MNCO, the incidence of neoplasms was breast, 40%; skin, 82%; kidney, 81%; and pancreas 19%. One of the pancreatic neoplasms was an invasive carcinoma. MNCL appears to be especially effective as a renal carcinogen, whereas MNCO is most effective as a skin and breast carcinogen. MNDABA appears to be an effective carcinogen for kidney, skin, and breast, and the most effective of the 3 on a molar basis in inducing atypical acinar cell changes in the pancreas. The compounds MNDABA, and MNCO thus induce lesions in the pancreas similar to those which we have noted previously with azaserine. It appears that these two compounds are as effective as azaserine in inducing preneoplastic and neoplastic pancreatic lesions; however, they suffer from the disadvantage of inducing a higher incidence of potentially lethal neoplasms in other target tissues (skin, kidney, breast) and thus provide a less satisfactory animal model of pancreatic cancer than does azaserine. They do provide a model for studying the effects of carcinogenic chemical in the pancreas.

Significance to Biomedical Research and the Program of the Institute: This approach has yielded a biological model of pancreatic carcinogenesis in which cancers appear to arise in acinar cells. Comparison of results with several agents should help elucidate the importance of pancreatic localization of chemical structure as determinants of pancreatic carcinogenesis by chemicals. Such information will form one basis for recognizing potential pancreatic carinogens in the environment.

Proposed Course: Work on development of animal models of pancreatic carcinoma under this contract will conclude during the current year.

Date Contract Initiated: June 28, 1972

Current Annual Level: $0

DOE-NCI INTERAGENCY AGREEMENT (BROOKHAVEN NATIONAL LABORATORY (YOI-CP-60219)

Title: Influence of Repeated Low Dose Irradiation on Mammary Gland Carcinogenesis in Estrogenized Rats

Contractor's Project Director: Dr. Claire J. Shellabarger

Project Officer (NCI): Dr. D. Jane Taylor

Objectives: It has been demonstrated that a synergistic interaction occurs between x-irradiation and estrogen on mammary adenocarcinoma formation in female rats of the AxC strain, and so far as is known, only in the AxC strain. It is the objective of this contract to study the effect of fractionation and protraction of the x-irradation on this synergistic interaction.

Major Findings: Female rats of the AxC strain are required, and since they were not commercially available in large numbers, the first effort of this contract has been to breed the required number of rats. All rats needed for the tumorigenic portion of the study have been produced and entered into the study. A pilot experiment has suggested that female rats of the Fischer strain do not show a synergistic interaction between x-irradiation and DES. Consequently, this strain cannot be used to accomplish the objectives of this contract.

Significance to Biomedical Research and the Program of the Institute: Because it has been reported that women exposed to repeated fluoroscopic examinations, women survivors of atomic bombings and possibly women treated with x-ray for acute postpartum mastitis, all have an increased risk of developing breast cancer, radiation exposure is now associated with breast cancer of the human female. However, there is little animal data on the dose-response relationship of radiation and the risk of developing mammary neoplasia at low doses of radiation and even less data on the possible sparing effect when the radiation is fractionated and protracted. It is the intent of this research to investigate the dose-response relationship and the effect of fractionation in an animal model in which synergistic interaction between x-rays and estrogen on mammary carcinogenesis has been demonstrated.

Proposed Course: The incidence of rats with mammary adenocarcinomas will be related to the dose of radiation and to the schedule of radiation and correlated with prolactin levels.

Date Contract Initiated: September 30, 1976

Current Annual Level: $177,090

DOE-NCI INTERAGENCY AGREEMENT (BROOKHAVEN NATIONAL LABORATORY) (Y01-CP-30213)

Title: Synergistic Interaction of Hormones and Neutron Radiation on Mammary Gland Carcinogenesis

Contractor's Project Director: Dr. Claire J. Shellabarger

Project Officer (NCI): Dr. D. Jane Taylor

Objectives: To determine if the synergistic interaction between x-irradiation and estrogen on mammary gland carcinogenesis in the female AxC rat can also be observed for neutron radiation; and to understand the mechanism of such synergism.

Major Findings: The reported synergistic interaction between diethylstilbestrol (DES) and x-rays on mammary adenocarcinoma formation in AxC female rats has been found to hold also for DES and neutrons. This synergism does not obtain when female rats of the Sprague-Dawley strain are studied. DES has been found to induce much higher levels of serum prolactin in AxC rats than in Sprague-Dawley rats. Thus, prolactin is implicated in the synergistic interaction of DES and radiation on mammary adenocarcinoma formation. As the dose of DES is lowered in AxC rats, synergism with radiation is delayed and may even be absent. When the time between neutron radiation and DES treatment is lengthened, synergism of mammary adenocarcinoma formation is maintained suggesting that there is little or no recovery or repair of radiation injury.

Significance to Biomedical Research and the Program of the Institute: Because it has been reported that women exposed to repeated fluoroscopic examinations, women survivors of atomic bombings and possible women treated with x-ray for acute postpartum mastitis, all have an increased risk of developing breast cancer, radiation exposure is not associated with breast cancer of the human female. With more frequent use of neutron services in industry, research and medicine, the possibility of increased exposure of women to neutron-irradiation is expanded. Also, the use of steroids for contraceptive and/or replacement therapy has increased. Thus, the interactions of neutron-irradiation and sex steroids in regard to mammary carcinogenesis should be evaluated. This project is an attempt to develop an animal model, and to understand the model itself, so that the qualitative aspects of mammary carcinogenesis in man may be evaluated in terms of cause and prevention of breast cancer.

Proposed Course: The relative biological effectiveness of neutrons for mammary adenocarcinoma formation will be determined in female AxC rats both in the presence and absence of DES treatment.

Date Contract Initiated: June 28, 1973

Current Annual Level: $0

EXPERIMENTAL PATHOLOGY LABORATORIES, INC. (NCI-CP-65763)

Title: Resource for Microscopic and Autoradiographic Technology

Contractor's Project Director: Dr. Beverly Y. Cockrell

Project Officer (NCI): Dr. Curtis C. Harris

Objectives: The Contractor serves as a resource for preparation and examination of tissues both for high resolution autoradiography (1 micron sections of plastic-embedded tissues) and for high resolution light and electrol microscopy. Microscopy will be used to provide data on: (1) the pathogenesis of tumors of various target organs such as lung, colon, skin, pancreas, and prostate; and (2) the localization of labeled compounds, including carcinogens into cellular organelles by autoradiographic techniques.

Major Findings: Task I - This is a service task to provide quality thick sections of plastic embedded material.

Task II - III - Electron microscopy showed that sulfaguanidine given during the administration of CCl protected rats from the toxic effect of CCl .

Significance to Biomedical Research and the Program of the Institute: Electron microscopy and autoradiography have become valuable tools in the study of carcinogenesis. Service efforts in these areas hasten the advancement of studies within the Institute.

Proposed Course: Task I - The contractor's performance is on schedule with at least 75 slides a week being delivered to NCI.

Task II - III - Completed.

Date Contract Initiated: September 23, 1976

Current Annual Level: $34,320

GEORGIA, MEDICAL COLLEGE OF (NO1-CP-55656)

Title: Study of the Potential for Metabolic Activation in Animal Pancreas

Contractor's Project Director: Dr. Owen Black, Jr.

Project Officer (NCI): Dr. David G. Longfellow

Objectives: To determine if the pancreas in experimental animals possess the potential for metabolic activation or biotransformation of carcinogens and other agents.

Major Findings: High pressure liquid chromatography (HPLC) of 3-methylcholanthrene (3MC) metabolites from in vitro and in vivo studies has been completed. These studies disclosed that pancreatic metabolism of 3MC occurred by hydroxylation at both the 1,2-position and the 11,12-position of the molecule. Metabolism at the 1,2-position appeared to be the major pathway. In agreement with published information, HPLC of hepatic metabolites showed similar results. In vivo studies of the metabolism 7,12-dimethylbenzanthracene (DMBA) were completed except for HPLC identification of metabolites. There were several areas in which the in vivo metabolism of DMBA differed from the metabolism of 3MC. DMBA reached a peak of tissue content much sooner than 3MC and appeared to be metabolized to a greater extent and more rapidly than 3MC. The pancreatic uptake of DMBA, on a per gram tissue basis, was similar to that of the liver. Studies of the in vitro metabolism of benz(a)pyrene (BP) have been completed except for HPLC identification of the metabolites. In contrast to what was demonstrated for 3MC and DMBA, the metabolism of BP was enhanced by pretreatment with both 3MC and phenobarbital (PB). In vivo BP studies are underway. The initial studies indicate that uptake of BP per gram of tissue is greater for pancreas than for liver. Preliminary in vitro studies of the metabolism of methylnitrosourea (MNU) have indicated that the compound is too unstable for these in vitro metabolic studies. Published data indicate other analogs of MNU are equally unstable and would offer no advantage over MNU. The in vitro metabolism of 5,5-dimethyloxazolidine (2,4-dione) or DMO is currently being examined.

Significance to Biomedical Research and the Program of the Institute: Data from this project has shown that pancreatic tissue has the capability to biotransform precarcinogens to carcinogens, increasing the risk of this tissue for tumors. Moreover, it has also provided evidence that some carcinogens may be preferentially taken up by the pancreas, additionally increasing the risk of cancer for this tissue. Finally, contractor has shown that, except for its inducibility, the pancreatic metabolizing system resembles the hepatic system. These observations are important in determining the role that pancreatic biotransformation plays in carcinogensis of the tissue.

Proposed Course: Studies of the in vivo metabolism of BP will be completed; these will be followed by studies of MNU and DMO. After completion of in vitro studies on DMO, analysis of the metabolism of azaserine, dimethylaminoazobenzene, and diisopropanolnitrosamine will be undertaken.

Date Contract Initiated: July 1, 1975

Current Annual Level: $58,000

GEORGIA, MEDICAL COLLEGE OF (NO1-CP-43282)

Title: Epidemiological Study of Colon Cancer among Blacks and Whites

Contractor's Project Director: Dr. Warren H. Gullen

Project Officer (NCI): Dr. Carl E. Smith

Objectives: Investigate, via a case-control study in nine Atlanta hospitals, the etiological relationship between dietary factors and colon cancer with emphasis on the role of fiber, animal protein, saturated fats, and cholesterol.

Major Findings: None. Study is in data analysis phase.

Significance to Biomedical Research and the Program of the Institute: Fiber, animal protein, saturated fats, and cholesterol have all been implicated in the etiology of colon cancer, both in the scientific literature and the lay press. However, there is a paucity of real information, and what information there is tends to be contradictory and certainly not definitive. Therefore, the Institute initiated this study (as well as others) of American population groups with a lower than average incidence of cancer of the colon (colon cancer is less common in the South than in the North and less common in Southern blacks than in Southern whites).

Proposed Course: The execution phase, which is completed, yielded 210 case-control pairs. The data will be analyzed to investigate dietary (and other) differences between the cases and controls.

Date Contract Initiated: February 1, 1974

Current Annual Level: 0

HARVARD UNIVERSITY SCHOOL OF PUBLIC HEALTH (NO1-CP-33273)

Title: Factors Influencing Experimental Respiratory Carcinogenesis by Alpha Radiation and Chemical Carcinogens

Contractor's Project Director: Dr. John B. Little

Project Officer (NCI): Dr. Carl E. Smith

Objectives: To examine some of the factors which influence the carcinogenic response of respiratory carcinogens, to develop appropriate models for studying interactions between these agents, to determine whether a

synergistic or potentiating effect exists between alpha radiation and chemical carcinogens in the production of lung cancer, and to investigate conditions which may predispose to or be associated with such synergistic interactions. Polonium-210 (^{210}Po) will be used as the source of alpha radiation, and benzo(a)pyrene (BP) as the chemical carcinogen.

Major Findings: Lung cancer has been induced in hamsters by the intratracheal instillation of either ^{210}Po or BP adsorbed onto Fe_2O_3 carrier particles. No tumors occurred in untreated or Fe_2O_3-treated control animals. Distribution studies with ^{210}Po administered either in saline solution or on various concentrations of carrier particles indicate that the uniform distribution of alpha-emitting radionuclides as occurs following saline instillaton is equally or more carcinogenic as is the non-uniform distribution or "hot-spots" seen with the particulate instillation. Tumors have been induced in about 10% of hamsters receiving alpha radiation doses of only 15-75 rads from ^{210}Po. A significant synergistic interaction between low doses of ^{210}Po and BP appeared to occur when BP exposure followed ^{210}Po exposure by 4 months. Most of this effect could be ascribed, however, to a potentiating effect of subsequent saline instillations on ^{210}Po carcinogenesis. Preliminary data suggest that the saline instillations act to stimulate cell proliferation in the lung. These results emphasize the fact that seemingly innocuous stimuli may significantly potentiate lung carcinogenesis, an observation which may have important implications in terms of the interactions between alpha radiation and cigarette smoke in human populations.

Significance to Biomedical Research and the Program of the Institute: The proposed studies are addressed to a very critical problem in the evaluation of human lung cancer hazards which may result from the combined effects of radiation and chemical carcinogens. Synergistic relationships between alpha radiation and chemical carcinogens have been implicated in the high incidence of occupational lung cancer in man (uranium, hematite, and flurospar miners). Previous studies have shown the ^{210}Po, a component of tobacco smoke, accumulates in the bronchial wall, especially the segmental bifurcations of smokers. This is a region where a high percentage of the bronchial carcinomas are found. The possible role of ^{210}Po, acting as a cofactor along with other chemicals in tobacco smoke, needs clarification. Results from this project could have a direct bearing on the evaluation of lung cancer hazards in man, and on future developments in the whole Lung Cancer Program.

Proposed Course: Studies will be continued to assess synergistic effects between Po alpha radiation, BP and saline instillations following sequential administration, and to evaluate the importance of cell proliferation in such interactions. In addition, the effect of dose rate on the induction of lung cancer by alpha radiation will be studied following moderate and low total radiation doses.

Date Contract Initiated: June 1, 1973

Current Annual Level: $53,000

HAWAII, UNIVERSITY OF (N01-CP-75933)

Title: Synthesis of New Retinoids for In Vitro Studies of Prevention of Lung Cancer and Other Epithelial Cancers

Contractor's Project Director: Dr. Robert S. H. Liu

Project Officers (NCI): Dr. Carl E. Smith
Dr. Michael B. Sporn

Objectives: The purpose of this program is to prepare sufficient quantities of pure retinoids of different shape (geometric isomers), and to submit them to NCI for screening tests with the hope that a retinoid, least toxic and yet most effective in prevention of carcinogenesis will be identified.

Major Findings: In the past five years, research work carried out in the laboratory of Dr. Liu has led to the preparation of four new types of vitamin A (new geometric isomers) which due to the unusual shape (the 7-cis geometry) were earlier thought not possible to exist. Yet, Dr. Liu and co-workers found that such compounds once prepared are quite stable. Since preliminary results obtained at NCI seem to suggest that the shape of retinoids (which are modified forms of vitamin A) has an effect on their toxicity and effectiveness in cancer prevention, it becomes logical to prepare sufficient quantities of such new retinoids for screening tests.

Significance to Biomedical Research and the Program of the Institute: This project will be a part of a general program of the NCI to find new and alternate approaches to solve the problem of epithelial cancer. The emphasis is on prevention. Results already obtained suggest that cancer prevention is possible provided that a non-toxic retinoid can be found.

Proposed Course: The new geometric isomers of vitamin A will be prepared according to procedures in this laboratory. The method, however, has led to a mixture of different vitamin A isomers. In the past, due to separation difficulties only very small quantities of pure compounds (milligram quantities) were isolated. For meaningful screening tests, a much larger amount is needed. Fortunately, a new isolation method has since been developed, preparative high pressure liquid chromatography. Isolation of pure intermediates for extended synthesis, and purification of final products are expected to be much facilitated by the unit.

Date Contract Initiated: September 30, 1977

Current Annual Level: $40,837

HOUSTON, UNIVERSITY OF (N01-CP-75935)

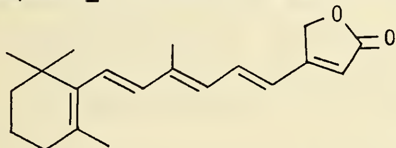
Title: Synthesis of New Retinoids for In Vitro Studies of Prevention of Lung Cancer and Other Epithelial Cancers.

Contractor's Project Director: Dr. Steven C. Welch

Project Officers (NCI): Dr. Carl E. Smith
Dr. Michael B. Sporn

Objectives: The objectives of this contract are to synthesize possible retinoid metabolites and retinoid analogues for submission to the National Cancer Institute for in vitro screening. Hopefully some of the retinoid derivatives will have the desired therapeutic indexes and/or pharmacokinetics (proper tissue distribution) to act as potential epithelial cancer chemopreventive agents.

Major Findings: During the past five months (October 1, 1977 to March 31, 1978), retinoid compound 1, a possible metabolite, has been prepared, as well as analogues 2 (R=C_6H_6), 3 (R=C_6H_6), and 4. Both compounds 2 and 3 were found to be inactive in 5/5 cultures at 10^{-9} M; however, compound 3 was found to be active in 4/5 cultures at 10^{-8} M.



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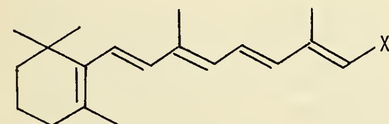


	X
2	$-CH_2SR$
3	$-CH_2SeR$
4	$-COCH_2SOCH_3$

Significance to Biomedical Research and the Program of the Institute: If a possible active retinoid metabolite or analogue (by in vitro screening) with the desired therapeutic index and pharamacokinetics (by in vivo screening) can be synthesized, then a reduction in the incidence of invasive epithelial cancer in human beings might be attainable. Drugs of this type would in effect act as cancer chemopreventive agents.

Proposed Course: Syntheses of retinoid derivatives will be continued with submission of each new compound to the National Cancer Institute for in vitro screening.

Date Contract Initiated: September 30, 1977

Current Annual Level: $65,531

IIT RESEARCH INSTITUTE (NO1-CP-75953)

Title: Studies of Colon Carcinogenesis in Organ Culture of Intestinal Mucosa

Contractor's Project Director: Dr. Leonard J. Schiff

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The objective of this project is the development of new methods for organ culture of colonic mucosa, so that it will be possible to study agents and mechanisms involved in carcinogenesis. Its specific aims are to: 1) optimize organ culture conditions using rat colon so the duration of in vitro experiments can be extended beyond a few weeks; 2) investigate, in organ culture, various aspects of lesion development and metabolism subsequent to treatment with carcinogens, co-carcinogens and promoters; 3) monitor lesion development and metabolism in explants of rat colon exposed in vitro and transplanted to syngeneic host; 4) investigate lesion development and metabolism of colonic explants immediately transplanted to host rats and subsequent treatment with carcinogens; and 5) monitor cultures exposed in vitro and after transplantation to host rats for in vivo neoplastic transformation.

Major Findings: Optimal conditions for maintenance of adult rat colon in organ culture are being studied. Culture techniques used during these experiments include a human fibrin foam matrix model and a grid technique using a membrane filter on a stainless steel platform. Maintenance of adult descending colon from antibiotic-treated conventional reared rats was established for 7 to 14 days with preservation of viability. Parameters measured were glycoprotein synthesis, incorporation of tritiated thymidine and leucine into DNA and protein, and morphological endpoints. The colonic mucosa during this period did not maintain its characteristic in vivo morphology; however, normal columnar epithelium did line the modified glandular crypts. These results were obtained when colonic explants were cultivated in Waymouth's MB 752/1 medium supplemented with 10% homologous rat serum or fetal bovine serum, 0.2% bovine serum albumin, ascorbic acid, hydrocortisone, insulin and ferrous sulfate. Cultures were incubated at 37°C in 5% CO_2-air.

Significance to Biomedical Research and the Program of the Institute: Cancer of the colon is currently a leading cause of cancer-related deaths in the United States. Nevertheless, certain phases of carcinogenesis research require a tool that does not involve the complexities of the whole animal. Since many carcinogenesis studies deal with cancerous behavior of a heterogeneous cell population, the organ culture methodology offers a unique opportunity for these studies.

The use of human fibrin foam as a matrix for three-dimensional organ cultures of rat colon tissue offers an improved model for in vitro studies -- one that combines preservation of histological architecture

both in the explant and outgrowth cells. By optimizing organ culture conditions, investigations into lesion development and metabolism can be made. Since the majority of colorectal carcinogenesis information has been obtained from studies using laboratory rats, emphasis of this program will be placed on the development of rat colonic organ culture methods that can be combined with transplantation into appropriate host animal. With this approach, contractor hopes to obtain more information concerning the mechanisms of chemical carcinogen/host tissue interaction.

Proposed Course: Experiments are continuing to optimize organ culture conditions using descending rat colon so that the duration of in vitro experiments can be expanded to approximately 3 to 4 weeks. These investigations will focus on the effect of various oxygen concentrations on long-term maintenance. In addition, the effects of exposing adult colonic epithelium in organ culture to chemical carcinogens, co-carcinogens and promotors will be investigated.

Date Contract Initiated: September 30, 1977

Current Annual Level: $75,755

ITT RESEARCH INSTITUTE (N01-CP-75939)

Title: Long-Term Studies of Prevention of Epithelial Cancer by Retinoids

Contractor's Project Director: Dr. Richard C. Moon

Project Officers (NCI): Dr. Carl E. Smith
Dr. Michael B. Sporn

Objectives: The major goal of this project is to assess the chemopreventive activity of retinoids (natural and synthetic analogues of vitamin A) in long-term studies conducted in rodent models for breast cancer.

Major Findings: Previous work has indicated that retinyl acetate suppresses the appearance of mammary adenocarcinomas induced in Sprague-Dawley female rats by 1-methyl-1-nitrosourea (MNU) or 7,12 dimethylbenz-(a)anthracene (DMBA). In ongoing long-term carcinogenesis studies, retinyl methyl ether, retinyl butyl ether and axerophthene have suppressed the appearance of palpable mammary tumors induced in Sprague-Dawley female rats by MNU. However, since these studies are still in progress, confirmation of the chemopreventive activity of these retinoids against mammary carcinogenesis must await the histopathological evaluation of the palpable tumors subsequent to the termination of the studies.

Significance to Biomedical Research and the Program of the Institute: Studies performed under this contract, although still in progress, indicate that efforts to synthesize organotrophic retinoids with increased chemopreventive activity and diminished toxicity in comparison to previously tested compounds, are meeting with success. The data

obtained from long-term evaluation of retinoids will hopefully lead not only to the establishment of the concept of cancer chemoprevention, but will also provide evidence for the use of retinoids in suppressing progression of early neoplastic lesions in women who are at high risk for breast cancer.

Proposed Course: Newly synthesized retinoids will be evaluated for chemopreventive activity in rodent models for breast cancer.

Date Contract Intiated: September 30, 1977

Current Annual Level: $123,000 (Supported by Carcinogenesis Testing Program)

ITT RESEARCH INSTITUTE (NO1-CP-43289)

Title: Supply of Animals Treated with Epithelial Carcinogens

Contractor's Project Director(s): Dr. Clinton J. Grubbs
Dr. Richard C. Moon

Project Officer (NCI): Dr. Michael B. Sporn

Objectives: The objective of this program is to supply animals with preneoplastic and/or neoplastic lesions of the respiratory tract, urinary bladder, esophagus and mammary gland to those organizations designated by the Project Officer.

Major Findings: A major effort has been directed toward the development and evaluation of a new method for inducing neoplastic lesions of the respiratory tract. The technique permits the induction of a cancer in a circumscribed region of the hamster trachea which is similar to bronchogenic carcinoma in humans. A catheter was fabricated which delivers the carcinogen (N-methyl-N-nitrosourea) to approximately a 5mm length of the trachea 10-15mm distal to the vocal cords. The treatment of 7-week-old hamsters 1X/week for 15 consecutive weeks with either 0.5 or 0.25% MNU induced a 68% and 33% incidence of tracheal cancers, respectively, within 6 months. This regimen appears ideal since it permits the treatment of a large number of animals without toxic effects. Additional studies using female hamsters, the carcinogen N-ethyl-N-nitrosourea, or various dosing schedules with MNU were not as effective due to either high mortality or lack of cancer induction. A new catheter tip has been constructed which will increase the area for reabsorption of the carcinogen by a factor of four and will prevent mechanical damage to the trachea during insertion. Hamsters treated for various periods of time with 0.5% MNU, as well as tracheal cytological samples from such animals, were provided to investigators designated by NCI. Over 500 cytology samples from 290 animals were shipped during the previous year at various intervals. Animal models for cancer of the mammary gland

(dimethylbenz(a)anthracene and MNU), urinary bladder (N-butyl-N-4-hydroxybutylnitrosamine), and esophagus (methylbenzylnitrosamine) have also been utilized to supply 3700 rats and mice to investigators designated by the Project Officer for studies relative to epithelial carcinogenesis in these organs.

Significance to Biomedical Research and the Program of the Institute: This contract will provide investigators with animals treated with several epithelial carcinogens for either in vivo or in vitro carcinogenesis experiments.

Proposed Course: To provide carcinogen-treated animals to investigators designated by the Project Officer and to continue to refine and improve existing epithelial tumor models in order to provide animals with preneoplastic and neoplastic lesions which are reproducible and rapidly induced.

Date Contract Initiated: May 20, 1974.

Current Annual Level: $176,000

ITT RESEARCH INSTITUTE (NO1-CP-23292)

Title of Project: Studies of Modulating Factors in Epithelial Carcinogenesis

Contractor's Project Director: Dr. Richard C. Moon

Project Officer (NCI): Dr. Michael B. Sporn

Objectives: The major goal of this project is to assess the chemopreventive activity of retinoids (natural and synthetic analogues of vitamin A), in animal models for epithelial cancers that are of major importance in human population.

Major Findings: Mammary Carcinogenesis. Retinyl acetate and retinyl methyl ether have been shown to suppress the appearance of mammary adenocarcinomas induced in Sprague-Dawley female rats by either 1-methyl-1-nitrosourea (MNU) or 7,12 dimethylbenz(a)anthracene (DMBA). In studies in which either a low or high incidence of cancers were induced, these retinoids significantly prolonged the latency of cancer appearance and reduced the average number of cancers per rat. Furthermore, it was demonstrated that continual dietary supplementation with retinyl acetate was necessary to maintain retinoid chemopreventive activity and that retinyl acetate appeared to exert its effect by inhibiting the progression of early neoplastic lesions. The trimethylmethoxyphenyl analogues of either retinyl methyl ether, retinoic acid ethyl amide, or retinoic acid ethyl ester and 13-cis-retinoic acid were found to be ineffective in inhibiting mammary carcinogenesis induced by MNU and/or DMBA.

Urinary Bladder Carcinogenesis. Animal models have been developed for use in the study of transitional cell and epidermoid carcinomas of the urinary bladder in rats and mice, respectively. A dose-dependent induction of urinary bladder cancer with N-butyl-N-(4-hydroxybutyl)-nitrosamine (OH-BBN) using a quantative dosing schedule has been achieved. In Fischer rats feeding 13-cis-retinoic acid after completion of carcinogen treatment, diminished the number and severity of cancers and other proliferative lesions of the urinary bladder. Similarly, feeding of 13-cis-retinoic acid to C57BL/6 male mice after completion of OH-BBN treatment prevented the development of both cancers and benign tumors.

Esophageal Carcinogenesis. Subcutaneous administration of methylbenzyl-nitrosamine has been shown to induce esophageal cancers in female Fischer 344 rats in a dose dependent manner. Aromatic analogues of retinoic acid, namely the trimethylmethoxyphenyl analogues of either retinoic acid ethyl ester or retinoic acid ethyl amide, were found to have no significant chemopreventive activity against the induction of esophageal cancers in this tumor model system.

Respiratory Carcinogenesis. A reproducible method of inducing carcinomas in a localized area of the tracheobronchial epithelium of the Syrian Golden hamster has been developed. Induction of cancer by 1-methyl-1-nitrosourea has been demonstrated to be dose dependent and intratracheal instillation of 0.5% MNU one time per week for 15 weeks has induced a high cancer incidence. This cancer model is now being used for the evaluation of the chemopreventive activity of the retinoids, 13-cis-retinoic acid and 4-hydroxyphenyl retinamide.

Significance to Biomedical Research and the Program of the Institute: Studies performed under this contract have led to the development of new techniques for the safe but rapid induction of epithelial cancers in rodents. These models have not only provided suitable systems for the evaluation of anti-cancer agents, but may also provide techniques by which other compounds can be evaluated for potential carcinogenicity to man. The data obtained from the evaluation of retinoids in various epithelial cancer models will hopefully lead to the development of structure-function relationships for retinoids as related to carcinogenesis, as well as permit the establishment of the new concept of chemoprevention.

Proposed Course: Newly synthesized retinoids will be evaluated for chemo-preventive activity against respiratory, breast, urinary bladder, and esophageal cancer. In addition, the refinement and improvement of epithelial tumor models will be continued.

Date Contract Initiated: June 26, 1972.

Current Annual Level: $480,000

ILLINOIS, UNIVERSITY OF (N01-CP-65843)

Title: Maintenance and Scheduled Sacrifice of Guinea Pigs

Contractor's Project Director: Dr. Samuel S. Epstein

Project Officer (NCI): Dr. Carl E. Smith

Objectives: This project was designed primarily to investigate the carcinogenic potential in F_1 progeny of Hartley guinea pigs of maternal transplacental exposure to N-nitrosomethylurea (NMU) and N-nitrosomethylurethane (NMUT); to investigate the carcinogenic effects of NMU and NMUT, in male Hartley guinea pigs following partial pancreatectomy; and to investigate the possible in vivo biosynthesis of NMU, and its carcinogenic effects following administration of methyl guanidine and sodium nitrite to guinea pigs. Studies were also undertaken to investigate DNA repair in guinea pig pancreatic slices, in vitro, and in the pancreas, in vivo, following exposure to NMUT and 4-hydroxyaminoquinoline-1-oxide (HAQO).

Major Findings: No exocrine pancreatic tumors have so far been induced in contractor's carcinogenesis studies during the past year. However, pancreatic islet cell tumors were found in 7 out of 678 guinea pigs (1%) that survived more than 2 years. These guinea pigs, both random-bred Hartley and inbred strain 13, were involved in various carcinogenicity studies with NMU and NMUT. Four tumors were observed in a total of 374 carcinogen-treated animals (1.1%) and 3 tumors out of 304 controls (1.0%), and there does not appear to be any significant relationship with carcinogen treatment. The incidence was higher in the inbred strain 13 (3 out of 85, 3.5%) than in random-bred Hartley guinea pigs (4 out of 593, 0.7%). These tumors were apparently spontaneous, histologically varied and, except in one case, were beta-cell adenomas.

Additionally, 3 uterine tumors (latency period 42-44 weeks) were induced in the female F_1 progeny of guinea pigs which received NMUT during pregnancy, and one uterine tumor (latency period 46 weeks) in the control F female group of guinea pigs which received NMUT during pregnancy.

Intragastric administration of NMU to strain 13 male guinea pigs, at a weekly dosage of 7.5 mg/kg for 15 weeks and then twice weekly for a subsequent 15 weeks, induced high toxicity, as evidenced by weight loss and mortality and a high incidence of malignant neoplasms, over a total observational period of 40 weeks. The neoplasms included hepatic angiosarcomas, cholangiocarcinomas, and generalized lymphoblastic lymphomas.

The nature of DNA damage induced by NMUT in the guinea pig pancreas, both in vitro and in vivo, and subsequent repair was further investigated by alkaline sucrose density gradient analysis, using a non-radioactive fluorimetric procedure for DNA determination in gradient

fractions. In vitro exposure of pancreatic slices to 20 mM NMUT for 30 min damaged DNA to less than 2.24 x 10^6 dalton fragments. However, incubation of NMUT-treated slices for 3 h in a fresh medium resulted in repair of most of the DNA damage, as indicated by the conversion of low molecular weight DNA fragments into heavy DNA of molecular weight comparable to DNA from control slices. Additionally, a single administration of NMUT (30 mg/kg, i.p.) to guinea pigs induced extensive DNA damage, to less than 2.24 x 10^6 dalton fragments in the pancreas within 4 h; similar DNA damage was observed in the liver. However, in the pancreas and liver of guinea pigs sacrificed at increasing intervals after NMUT administration, there was a gradual conversion of shortened DNA fragments to heavy high molecular weight DNA, indicating repair of DNA damage. It appears that most of the DNA damage in the pancreas and liver was repaired by 14 and 7 days, respectively, following NMUT administration.

Significance to Biomedical Research and the Program of the Institute: These studies were designed to develop an animal model to investigate the carcinogenesis of pancreatic tumors, and the underlying biochemical mechanism of carcinogen-nucleic acid interactions in the pancreas. Such studies are considered to be basic to the etiology of pancreatic cancer.

Proposed Course: This was the final year of the project; contract ends 5/29/78.

Date Contract Initiated: September 30, 1976

Current Annual Level: 0

INDIANA, UNIVERSITY OF (NO1-CP-55654)

Title: Chemical and Structural Requirements for Pancreas Uptake and Excretion: Rats and Guinea Pigs

Contractor's Project Director: Dr. Roger P. Maickel

Project Officer (NCI): Dr. Carl E. Smith

Objectives: To examine the uptake, retention and excretion of a variety of organic compounds by the pancreas of rat and guinea pig, with the intent of characterizing the processes in terms of chemical structures and/or physicochemical properties.

Major Findings: An experimental system was developed, based on perfusion of the common bile duct in the rat, to permit collection of pancreatic exocrine secretions at intervals over the period 0-90 minutes after i.v. administration of a labeled compound. Amylase activity was measured as a reference of pancreatic function; specific

analytical methods were used to assay compounds and metabolic product(s). For this test system, decamethonium, a bis-quaternary amine with only slight lipid solubility, had half-lives in plasma, pancreas, and pancreatic secretions of 25, 84, and 36 minutes, respectively. Among a series of basic compounds with moderate lipid solubility, D-amphetamine had half-lives of 33, 81, and 52 minutes; D-methadone had half-lives of 47, 78, and 86 minutes; L-methadone had half-lives of 74, 103, and 92 minutes; and propranolol had half-lives of 40, 122, and 28 minutes for plasma, pancreas, and pancreatic secretions, respectively. Thus, as compared to the half-life decay in plasma, the pancreas acted as a selective filter for these basic compounds. In addition, propranolol reduced pancreatic secretory activity (as measured by amylase secretion rates) at doses as low as 2 Mmoles/kg, i.v.

Significance to Biomedical Research and the Program of the Institute: The development and application of this test system seems to be a practical procedure for describing the handling of foreign compounds by the mammalian pancreas. Compounds showing extended half-lives in pancreas or pancreatic secretions, as compared to the plasma t1/2 may well be localized in pancreatic structures. Such findings could lead to retrospective studies to see if patients afflicted with pancreatic tumors bear a history of exposure to substances showing a predilection for unusual pancreatic retention.

Proposed Course: Two areas of research will be pursued, based on logical extensions of the data obtained to date.

One effort will concentrate on an attempt to develop a system for the isolated perfused pancreas, utilizing either the rat or guinea pig pancreas, with both systems tested if possible. Drug uptake, retention, and secretion will be performed in the perfused organ using compounds previously identified as having interesting pharmacokinetic aspects (amphetamine, decamethonium, methadone, propranolol).

In addition, extensive efforts will be devoted to examination of the localization of drugs and metabolites in subcellular fractions of the rat and/or guinea pig pancreas following acute (single dose) and chronic (repetitive dosage) administration.

Date Contract Initiated: June 30, 1975

Current Annual Level: 0

KENTUCKY, UNIVERSITY OF (NO1-CP-75954)

Title: Studies of Carcinogenesis in Human Tissues

Contractor's Project Director: Dr. H. Earle Swim

Project Officer (NCI): Dr. Curtis C. Harris

Objectives: The objectives of this project are: a) to develop methods for the preparation of primary cultures of human bladder and prostate; b) to establish conditions for the isolation of epithelial cells in mixed populations with fibroblasts; c) to determine the nutritional and other environmental factors required for the serial propagation of human epithelial cells for prolonged periods; d) to study the metabolism of various classes of carcinogens by epithelial cells; e) to test ability of chemicals to transform human epithelial cells; and f) to make strains of human epithelial cells available to other investigators.

Major Findings: Organizational details have been worked out with various services of the University Hospital to obtain a variety of human tissues which are suitable for culture. Major emphasis has been placed on studies of the bladder. Four of the 5 specimens obtained through the kidney transplant program have been successfully cultured in vitro. Data obtained from studies of the first 3 specimens provided a basis for the development of a method which provides a high yield of viable cells from the bladder. These cells have been serially propagated on several different media and the data so far indicate that either CMRL 1066 or S77 supplemented with 10 per cent fetal calf serum is satisfactory. Cells from different passages have been stored in liquid nitrogen. The bladder cultures contain a mixed population of epithelial cells and fibroblasts on the basis of morphology. Experiments are currently underway to establish conditions which favor the growth of the epithelial cells.

Significance to Biomedical Research and The Program of the Institute: The most obvious means of control of chemical carcinogens is through reduced exposure, but in a society where potential carcinogens are widespread, measures to reduce exposure require confirmation that compounds which are carcinogenic for animals are also carcinogenic for human tissue. Further, this approach provides a unique opportunity for linking investigations using experimental animals with human cancer.

Proposed Course: During the next project period emphasis will be placed on experiments designed to isolate epithelial cells from mixed populations with fibroblasts. D-Amino acids and analogs of L-proline will be tested for their ability to selectively interfere with the growth of fibroblasts. Studies of nutrition will be conducted to develop media which enhance the growth rate of epithelial cells. Improved media would facilitate the isolation of epithelial cells by cloning techniques.

Efforts will be continued to improve the tissue retrieval aspects of the program which should facilitate the initiation of studies of additional target tissues.

Date Contract Initiated: September 30, 1977

Current Annual Level: $117,025

LITTON BIONETICS, INC. (NO1-CP-65847)

Title: Resource for Microscopic and Autoradiographic Technology

Contractor's Project Director: Dr. Elizabeth W. Kingsbury

Project Officer (NCI): Dr. Curtis C. Harris

Objectives: The purpose of this contract is to provide electron microscopic and autoradiographic services to investigators in the Chemical Carcinogenesis Program as required, based on a task orientation.

Major Findings: Work in progress involves examining tissue culture explants, outgrowths and subcultivations of human trachea or bovine pancreatic duct. The types of cells isolated and the purity of clonal populations are examined by light and electron microscopy. Human bronchial cells are examined for the presence of specific markers such as cilia, mucus and keratin, and for more non-specific markers such as tight junctions, desmosomes, tonofilaments, etc.

Significance to Biomedical Research and the Program of the Institute: In order to investigate the impact of an increasing amount and variety of chemicals in the environment which may affect the incidence of cancer, many scientific methods and tools must be employed. This contract provides one of these basic tools as a resource for various types of studies.

Proposed Course: To continue to provide the services stated, as required by NCI investigators.

Date Contract Initiated: September 21, 1976

Current Annual Level: $55,000

LITTON BIONETICS, INC (NO1-CP-43274)

Title: Studies of Carcinogenesis in Human Tissues

Contractor's Project Director: Dr. Marion G. Valerio

Project Officer (NCI): Dr. Curtin C. Harris

Objectives: To use an immunodeficient animal model, the nude athymic mouse, for long-term survival of xenografts in order to study the transformation of human tissues exposed to various carcinogens, co-carcinogens, and anticarcinogens.

Major Findings: A barrier sustained specific pathogen free facility has proven adequate to breed and maintain the athymic nude mouse for long-term studies. In this environment a normal life span of over two

years for the nude mouse has been achieved. It has also been established that the nude mouse will support long-term survival of human bronchial tissue xenografts, maintaining graft viability and normal morphology for over 400 days. Human bronchial, human colon, human esophagus, human pancreatic duct, and bovine pancreatic duct tissues are presently under study. The carcinogens being used include 7, 12-dimethylbenz(a)anthracene, amosite asbestos, N-methyl-N-Nitrosoguanidine, aflatoxin B_1, benzo(a) pyrene, polonium 210, and 4-nitroquinoline-N-oxide.

Significance to Biomedical Research and the Program of the Institute: The establishment of an animal host for human tissue xenografts which have been treated with carcinogens and/or anticarcinogens in vitro and/ or in vivo will provide a model more predictive of the effects of carcinogens and anticarcinogens on human tissues than extrapolation from an animal tumor or tissue culture model system. This system provides an opportunity to study chemical carcinogenesis in the target tissues for human cancer.

Proposed Course: Continue long-term testing of the effects of various carcinogens upon the human tissue xenografts implanted in the athymic nude mouse. To determine tumorigenic potential of human cells exposed to chemical and physical carcinogens in vitro.

Date Contract Initiated: February 1, 1974

Current Annual Level: $158,000

MARYLAND, UNIVERSITY OF (NO1-CP-75947)

Title: Isolation, Identification and Culture of Epithelial Cell Types from the Pancreas of Experimental Animals

Contractor's Project Director: Dr. Raymond T. Jones

Project Officer (NCI): Dr. Gary Stoner

Objectives: 1) to isolate large amounts of viable bovine pancreatic ductal epithelial cells by biophysical methods; 2) to identify the isolated epithelial cells as well as endothelial and fibroblastic cells by high resolution light microscopy, cytology, scanning and electron microscopy, biochemical, histochemical and immunological methods; 3) to maintain the isolated bovine pancreatic ductal epithelial cells in cell culture; and 4) to do the above with small amounts (a few million cells) of bovine pancreatic ductal cells.

Major Findings: Thus far, the major findings in the six months that the project has been in operation have included development of a new method for isolating pancreatic ductal epithelial cells which enables these cells to be separated free from underlying stroma and almost entirely free of fibroblasts. It is based on a novel method of digestion of perfused whole

bovine pancreas followed by flotation of sheets of cells rather than total enzymatic digestion which has been used in the past and which leads to significant fibroblastic contamination. This technique, in the future, should be applicable to human pancreas as well. One of the problems in studying pancreatic carcinogenesis in vitro is the small amount of tissue available from humans and this is one reason why the development of cell culture methods should foster experimentation in this field.

The isolated pancreatic ductal cells, when placed in dishes, incorporate ^{3}H thymidine and so far have been cultured up to 2 months. These cells at various time intervals are now being studied by light and electron microscopy. At the present time, also antisera are being evaluated which have been prepared for these cells using both immunofluorescence and immunoperoxidase techniques.

Significance to Biomedical Research and the Program of the Institute: Carcinoma of the pancreas is the fifth leading cause of cancer death in the United States and the development of model systems is, therefore, extremely important. Recently, methods have been developed in this laboratory to study both human and bovine pancreatic ducts in explant culture, to graft them into immune deficient animals and to study them by biochemical and morphological means. Studies are also in progress in this laboratory evaluating effects of carcinogens in vitro. In the pancreas, because of limited amounts of materials, it will be highly desirable to develop methods for propagation of cells from the pancreatic duct which can be stored at the time of collection and studied in a variety of ways at future times. Furthermore, the use of cell cultures will enable somewhat more refined studies on mechanism of carcinogenesis to be identified.

Proposed Course: Further characterization of the cells by a variety of markers including immunologic studies and light and electron microscopy is needed. These are presently underway. Long-term maintenance in subculture of the cells needs to be developed and evaluated and, hopefully, the methods can be scaled down to a few million cells for reasons mentioned above.

Date Contract Initiated: September 29, 1977

Current Annual Level: $48,760

MARYLAND, UNIVERSITY OF (NO1-CP-75909)

Title: Studies of Carcinogenesis in Human Tissues

Contractor's Project Director: Dr. Benjamin F. Trump

Project Officers: Dr. Curtis C. Harris

Objectives: The objectives of these studies in esophagus are: 1) to obtain human tissues following surgical resection and/or immediate autopsy; 2) to study the morphological and cytochemical characteristics of epithelium (removed at zero time) in normal, premalignant and malignant states; 3) to develop methods for long-term organ culture of human tissues; 4) to study themorphological and cytochemical characteristics of epithelium in normal, premalignant and malignant states maintained in organ culture; and 5) upon the successful completion of these objectives, to study xenotransplantation of target organ tissue and its response to carcinogens.

Major Findings: Prior to the award of this contract, procedures for obtaining normal and neoplastic esophageal tissues from surgical resection and immediate autopsies had been successfully implemented. In total to date, 13 cases have been collected, 5 from surgery and 8 from immediate autopsy. Baseline studies have been initiated using light and transmission electron microscopy. In all instances examined, squamous cell carcinoma of the esophagus was composed of clusters of tumor cells outlined by basal lamina which was not seen in between the tumor cells. Villous cytoplasmic processes projected into the intercellular spaces but true lumen was not found. Well-developed desmosomes and rough endoplasmic reticulum were numerous. Abundant tonofilaments and dispersed fine filaments were present. Most of the nuclei were pelomorphic and contained prominent nucleolic. In one tumor, continuity between glandular epithelium and neoplastic squamous cells was observed. The significance of this is not clear at present.

When sufficient tissue is available as with specimens obtained from immediate autopsy, areas from the proximal, middle and distal thirds of the esophagus are grown in organ culture. Morphological examination of these cultures using high resolution light and electron microscopy, TEM and SEM, is in progress. The surface ultrastructure of the superficial cells at zero time is characterized by densely packed microridges which frequently form whirls. Individual cells are separated from eah other by very prominent terminal bars. During culture, the surface ultrastructure is changing, in that the microridges are progressively replaced by short micorvilli. The terminal bars are less prominent. It has been possible to maintain normal esophageal tissue in organ culture, in some instances, for 6 months.

Significance to Biomedical Research and the Program of the Institute: There is a very important need for human studies in the area of carcinogenesis. An estimated 60% to 90% of human cancers are caused by chemical agents; yet, precise data are needed on metabolism and response of human tissues to carcinogens at the morphological and biochemical level. Organ culture systems of human epithelium are of great value in determing the biochemical, molecular and pathogenic mechanisms of action of carcinogen-target cell interaction.

Proposed Course: 1) Continue to obtain esophageal tissues from normal, premalignant and malignant esophageal tissues and to morphologically

characterize them using high resolution light and electron microscopy; 2) continue growth of esophageal tissue in long-term organ culture and its exposure to chemical carcinogens, 3) continue examination of these control and carcinogen - treated cultures as stated above in 1; and 4) initiate xenotransplantation studies in the nude mouse.

Date Contract Initiated: September 30, 1977

Current Annual Level: $32,204

MARYLAND, UNIVERSITY OF (NO1-CP-43237)

Title: Studies of Carcinogenesis in Human Tissues: Bronchial Epithelium, Pancreas, Breast and Colon.

Contractor's Project Director: Dr. Benjamin F. Trump

Project Officer (NCI): Dr. Curtis C. Harris

Objectives: 1) To acquire normal, preneoplastic and malignant human tissues and to study the morphological and cytochemical characteristics before, during and after growth in explant culture; and 2) To explore tissue responses to application of selected carcinogens; aryl hyrdocarbon hydroxylase (AHH) determinations are made in selected tissues.

Major Findings: Bronchus: 1) Morphological [including transmission and scanning electron microscopy, (TEM, SEM)] and histochemical studies of primary lung carcinomas are continuing. One hundred and twenty tumors have been examined and classified according to histogenetic criteria developed. The distribution of carcinoembryonic antigen is being studied in these tumors using an immunoperoxidase technique. Correlative cytological criteria have been defined and tumors can be classified histogenetically based on Papanicolau-stained preparations. Clinical histories are being extracted from hospital medical records and patients answer a questionnaire, so that correlative clinical studies can be made in the future. 2) Work is in progress correlating quantitative carcinogen binding to "normal" bronchial epithelium in vitro, with histogenetic tumor type and with epidemiological data. 3) Bronchial epithelium has been exposed to benzo(a)pyrene-Fe_2O_3 over a 2-month period in vitro. Epidermoid metaplasia and lesions resembling carcinoma in situ were seen in treated explants. The effects of the direct acting carcinogen N-methyl-N-nitroso-N-nitroguanidine (MNNG) are presently being studied. 4) Studies on regeneration of the hamster trachea have been extremely helpful in contractor's understanding and interpretations of metaplastic and neoplastic changes in human bronchial epithelium. 5) Induction of AHH in explant culture of human bronchus from nine immediate autopsy patients was studied by exposing the tissues to benzanthracene. Consistent induction of AHH above the control level was observed, which ranged from 2 to 17 fold, depending on the individual patient. Different culture media were tested for monocyte culture and it was found that Contractor's explant culture medium (CMRL-

1066) plus 10 mM HEPES buffer was most satisfactory. In this way both target tissue and peripheral blood monocytes can be cultured in identical media, eliminating the variability of culture conditions.

Pancreas: 1) Human ductal epithelium has been exposed in explant culture to MNNG. Cytological changes at the light and electron microscopic levels can be produced in explant culture, by exposure to MNNG, that closely resemble those occurring in human matastasizing adenocarcinomas and in the Syrian hamster model of pancreatic carcinoma. 2) Studies are in progress whereby human ductal explants, exposed in vitro to MNNG, are being transplanted subcutaneously into athymic (nude) mice. 3) The metabolism of benzo(a)pyrene and 7,12-dimethylbenzanthracene (DMBA) has been studied in explants of human pancreatic duct in a chemically defined medium. The human pancreatic duct has the capacity to activate these polynuclear aromatic hydrocarbons into metabolic intermediates that bind to DNA.

Breast: 1) Morphological examination of normal, dysplastic, premalignant and malignant tissues is continuing. Ninety-nine cases are now available for study. 2) Explant culture of normal and malignant epithelium is being studied. Normal human tissue can be readily maintained in explant culture for 3-6 months and shows characteristics of adeno and epidermoid differentiation. 3) Normal epithelium, obtained from immediate autopsy specimens, has been treated with either DMBA or MNNG in vitro. Within the central portion of the explants, hyperplastic areas were observed. The cells had a high nuclear to cytoplasmic ratio, aberrant nucleoli and marked scalloping of the ductule on the basal surface. The hyperplastic outgrowth of epithelial cells was markedly altered by the carcinogens. The cells in the outgrowth had random orientation and demonstrated adeno and epidermoid characteristics. 4) Collaboration with Dr. Douglas Janss at Frederick Cancer Research Center continued and control and DMBA treated-human and rat monolayer epithelial cell cultures have been studied. In addition, collaboration exists with Dr. Dallas Purnell, University of Maryland, comparing the in vivo DMBA rat model with the situation in the human.

Colon: 1) Morphological and histochemical studies of normal, premalignant and malignant human epithelium are continuing. A total of one hundred and twenty seven cases are now available for study. Most cases have been studied by light microscopy and many cases have been examined by TEM. About thirty percent of the cases have been examined by SEM. Complete evaluation of the morphological and cytochemical aspects of these tissues is in progress. 2) Normal colonic epithelium is routinely being grown in explant culture. Variations in culture media constituents, size of tissue and supportive materials have been tested. Small (2 mm) tissue pieces grown in CMRL 1066 media with high glucose and a gas mixture of 95% O_2-5%-CO_2 at 37°C, results in 7-8 day viability. 3) Studies of colon carcinogenesis in the rat induced in vivo and in vitro by azoxymethane are continuing. These studies, not supported by the contract, are very important in comparative studies with human tissues.

Signifance of Biomedical Research and the Program of the Institute: The importance of this project lies in the fact that the nature of premalignant changes in human bronchus, pancreas, colon and breast are not well understood and little is known concerning carcinogen binding and the response to carcinogens of human tissues. A number of observations have been made which ultimately are expected to offer to the study of carcinogenesis new tools and resources in its search for identification of the key events leading to induction of cancer. These studies, it is hoped, will be of significance in understanding the process of carcinogenesis and will establish models for future studies in these and other organ systems.

Proposed Course: a) To further determine the nature of the cellular response to carcinogens in the human bronchus; b) to further establish a classification of lung tumors and correlate with clinical findings and epidemiological factors; c) to examine the metabolic and morphologic response of human bronchus to carcinogens in vitro and in vivo and to correlate this response with smoking and occupational histories, as well as with carcinogen metabolism by other cells such as peripheral blood monocytes; and d) to perform similar studies on breast, pancreatic duct and colonic mucosa.

Date Contract Initiated: October 1, 1973.

Current Annual Level: $337,069

MAYO FOUNDATION (NO1-CP-55660)

Title: Reflux as a Mechanism for Induction of Adenocarcinoma of the Pancreas

Contractor's Project Director: Dr. Eugene P. DiMagno

Project Officer (NCI): Dr. Carl E. Smith

Objectives: 1) Examine at autopsy the anatomical relationships of the human pancreatic duct, bile duct and duodenum and relate this arrangement to duct cell histology; 2) determine if there are relationships between epidemiological variables such as sex, smoking habits, alcohol intake, lipoprotein patterns and duct cell histology; 3) develop an animal model for induction of pancreatic duct reflux; and 4) examine human pancreatic secretion for potential carcinogens.

Major Findings: As detailed in last year's annual report, procedures have been developed to anatomically and radiographically determine the anatomical relationships between the pancreatic duct, bile duct and duodenum, as well as examine pancreatic duct histology in human autopsy specimens. Information was also obtained regarding smoking and alcohol habits as well as serum triglycerides, cholesterol, routine laboratory data, occupation, sex, age and cause of death and all other diagnoses from the Clinic record.

To date, autopsies have been performed on 330 cases with analyses completed on 265. Results indicate that the incidence of abnormal histology is related to increasing age ($\underline{P} < 0.001$), respiratory disease (chronic obstructive pulmonary disease and pneumonia) and neoplastic causes of death ($\underline{P} < 0.002$). It was also noted that a trend toward increased frequency of abnormal duct histology appears to be present in those postmortem specimens with separate openings of the common bile duct and pancreatic duct ($\underline{P} = 0.007$) compared to other ductal entries into the duodenum (a well delineated ampulla, a common channel entry for the common bile duct and pancreatic duct without a definite ampulla and a well defined septum between the pancreatic duct and common bile duct with a common entry into the duodenum). The associations between pancreatic histology and causes of death as well as duct openings are independent of age and sex. Thus, it appears the relationship between ductal openings, the presence of other neoplasms or respiratory disease and age may be related to presence of abnormal pancreatic ductal epithelium suggesting that a combination of factors may be related to induction of pancreatic neoplasia. However, it should be stressed that these data are still rather preliminary and that more data is needed to establish firm statistical associations. In addition, an analysis of multiple variables such as cigarette smoking and alcohol and their significance to the production of abnormal pancreatic duct histology is underway.

The second major effort of this contract was to develop an animal model for induction of pancreatic duct reflux. This effort has been very successful: A canine model has been developed comprising a chronic, indwelling duodenal cannula and a permanent pancreatic duct-cutaneous catheter. With this model one can simultaneously quantify pancreatic enzyme outputs, duodenal volume flow and pancreatic and duodenal pressures under physiologic circumstances. In a study recently published from this laboratory, it was demonstrated that in 12 studies in four conscious healthy dogs, mean fasting pancreatic pressure was 5 to 10 cm higher than mean fasting duodenal pressure. Furthermore, 8 minutes after ingestion of the meal, both pancreatic and duodenal pressures increased. Suprisingly, the mean duodenal pressure was higher than the mean pancreatic pressure 20 minutes after eating.

This relationship lasted for 20 minutes. Lastly, elevation of postprandial pancreatic pressure occurred concomitantly with increased pancreatic enzyme output and duodenal pressure increased with increased duondenal volume flow. It was postulated that these observed large,postprandial duodenal volume flows associated with duodenal pressures greater than pancreatic duct pressures may favor reflux of duodenal contents into the pancreatic duct.

In subsequent studies conducted in the past year, it has been demonstrated that a nonabsorbable radioactive marker perfused through the duodenum can be recovered from pure pancreatic juice obtained through the indwelling pancreatic catheter. Although the total amount of duondenal contents refluxed in the pancreatic ducts is small and did not exceed 0.28% of the entire volume flow in the duodenum per sampling period, the timing of the

reflux corresponded with the pressure relationships found in the earlier studies. Thus, in the dog under physiologic conditions, duodenal contents appeared to reflux into the pancreatic duct.

As these studies progressed, it has become more readily apparent that factors influencing pressure gradients from the duodenum to the pancreatic duct and pancreatic sphincter behavior are probably a very important determinant for reflux into the pancreatic duct. Thus, efforts now continue utilizing the identical canine model described above to measure pancreatic duct pressures, duodenal pressures and pancreatic sphincter yield pressures under control conditions and after hormonal stimulation. In our initial studies, the hormone secretin was selected and administered at doses of 0.75, 1.5, and 3.0 U/kg/hr i.v. for 2-hr periods. In 14 studies performed in four dogs, the duodenal pressure and sphincteric yield pressures were significantly ($P < 0.05$) decreased by the highest dose of secretin used when compared to control values. In addition, pancreatic duct pressures decreased significantly ($P < 0.01$) in a dose response fashion with increasing doses of secretin. It was also observed that duodenal motor activity fronts were totally abolished by 3.0 U/kg/hr of secretin but were not altered by the two lower doses. Interestingly, after cessation of the 1.5 or 3.0 U/kg/hr of secretin, the duodenal motor activity fronts consistently reappeared within 15 to 30 minutes. These data demonstrate that secretin markedly decreases duodenal pressure, pancreatic duct pressure and sphincter yield pressure and abolishes activity fronts, particularly at doses greater than 1.5 U/kg/hr. Thus it has been demonstrated that hormones (in this case, secretin) do alter pressure relationships between the pancreatic duct and duodenum and have the potential for influencing reflux of duodenal contents into the pancreatic duct. In particular, it was noted that a dose of secretin (1.5 U/kg/hr) diminishes pancreatic duct pressures and yet does not influence duodenal motor activity fronts. During duodenal motor activity fronts, duodenal pressure does increase above pancreatic duct pressure and reflux from the duodenum into the pancreatic duct could potentially occur during this time.

Significance to Biomedical Research and the Program of the Institute: In autopsy studies, it is becoming increasingly apparent that multiple factors may be involved in human pancreatic carcinogenesis. One of these factors is the relationship of the pancreatic ductal and bile duct entries into the duodenum. Surprisingly, separate openings of these ducts rather than a common entry of the ducts may be an underlying important feature in pancreatic carcinogenesis. This may suggest that if reflux is important, contents refluxed from the duodenum itself rather than from just bile may be a possible mechanism for induction of pancreatic cancer. It is appropriate then that animal models which have separate openings for the pancreatic and biliary duct be studied to investigate mechanisms of reflux. Such an animal model has been developed in this laboratory and used to demonstrate that reflux does occur under physiologic circumstances. Although the amounts of duodenal contents refluxed into the pancreatic duct are small, chronic reflux of even small amounts of carcinogens,

particularly in persons possessing other possible etiologic factors, may be important in human pancreatic carcinogenesis.

Proposed Course: More autopsy material must be investigated to place our human autopsy data on a firmer statistical basis. In addition, since information on clinic records regarding smoking and drinking is incomplete, more data regarding these habits is being developed from patient next of kin. In the canine model, studies will continue employing hormones, drugs and measurements of duodenal and pancreatic pressures as well as sphincteric mechanisms to better delineate the existence and physiology of reflux into the pancreatic duct. Specifically, a study of cholecystokinin octapeptide has been initiated and a plan to use glucagon is being contemplated. Combinations of gastrointestinal hormones are also planned for use in future studies. In addition, drugs, particularly nicotine, will be employed to determine whether or not it alters the pressure relationships between the duodenum and the pancreatic duct and to determine whether or not it will influence or cause reflux of duodenal contents into the pancreatic duct. Duodenal phase III activity may be a factor in causing reflux of duodenal contents into the pancreatic duct. During fasting these complexes appear approximately every 80 to 120 minutes and last for 5 to 10 minutes. During these time periods, duodenal pressures often exceed 100 cm of water and intermittently are greater than 60 to 70 cm of water above pancreatic pressure. Contractor has noted that reflux occasionally occurs during fasting in their canine model, but it is unknown whether these are related to the occurrence of type III duodenal activity. It is also largely unknown as to what the hormonal and neural control of these complexes are and whether drugs such as nicotine might influence type III duodenal activity. Hence, the study of duodenal motor activity which contractor plans to institute is also a promising avenue for research which may lead to greater understanding of control and causes of reflux into the pancreatic duct.

Date Contract Initiated: June 30, 1975.

Current Annual Level: 0

MEDIZINISCHE HOCHSCHULE HANNOVER (NO1-CP-75972)

Title: The Development of the European Hamster as an Animal Model for Pancreatic Carcinogenesis

Contractor's Project Director: Dr. Ulrich Mohr

Project Officer (NCI): Dr. Carl E. Smith

Objectives: Since the European hamster proved itself to be a most sensitive model in the field of pulmonary carcinogenesis, it was thought worthwhile to examine its reaction to the proven pancreatic carcinogen, 2,6-dimethylnitrosomorpholine (2,6-DMNM), and the related compound, 2,6-dimethylnitrosopiperazine (2,6-DMNP). Such studies are aimed not only at the development of a more sophisticated animal model for pancreatic

carcinogenesis, but might also provide corroboration of pancreatic findings achieved in the Syrian golden hamster, and hence exclude the possibility of a species-specificity. It is also hoped to use the European hamster to clarify the effects of hibernation on the metabolic conversion rate of 2,6-DMNM and consequently upon pancreatic tumor development.

Major Findings: The investigations are still in their very early stages and little data as yet exists. However, macroscopic examination of animals treated with 2,6-DMNM has indicated multiple, small, white pancreatic nodules. In addition, a pronounced jaundice, particularly of the liver, has been observed in almost all animals examined. This strong toxic effect is believed to have resulted in the early death of those animals treated with the highest dosage (1/5 LD_{50}) of 2,6-DMNM.

Significance to Biomedical Research and the Program of the Institute: Observations in recent years have indicated rising rates of pancreatic cancer. If a better understanding of this type of cancer is ever to be achieved it is necessary to develop a viable animal model for a closer examination of suggested parameters affecting pancreatic carcinogenesis. Various characteristics of the European hamster (size, life-span, hibernation) suggest that if pancreatic tumors similar in type and incidence to those already observed in Syrian golden hamsters can be induced, then this animal model would permit a more detailed study of the mechanisms and pathogenesis of pancreatic cancer.

Proposed Course: Once the exact nature of the response of the European hamster to 2,6-DMNM and 2,6-DMNP has been established, future experiments will concentrate on the most suitable and effective compound for detailed examination of various possible parameters of pancreatic carcinogenesis. These will include among other factors, cigarette smoke and alcohol.

Date Contract Initiated: September 30, 1977

Current Annual Level: $108,120

MELOY LABORATORIES, INC. (NO1-CP-55613)

Title: Transplacental Carcinogenesis in the Old World Monkey Erythrocebus Patas

Contractor's Project Director: Dr. D. Lewis Sly

Project Officer(NCI): Dr. Jerry M. Rice

Objectives: This project is designed to provide a nonhuman primate model to demonstrate and study transplacental chemical carcinogenesis.

Major Findinngs: Pregnant monkeys exposed to ethylnitrosourea have shown that the period of maximal fetal susceptibility to this chemical appears to be early in gestation. Tumors have been seen most frequently

in offspring first exposed to ENU at approximately 30 days of gestation. These tumors have had short latencies with most developing before six months of age. Tumors from animals exposed later in gestation have occurred later in life and with lower frequency. The tumor types seen in these transplacentally exposed primated differ markedly from the tumors seen in rodent studies. Sarcomas of various organ systems have been the primary finding, rather than carcinomas as seen in mice or neurogenic tumors as in rats. In addition, embryonal kidney tumors and hepatocellular carcinomas which produce α-fetoprotein have been induced transplacentally. Tumors have also been seen in juvenile and less frequently in pregnant female animals inoculated with ENU. Furthermore, preeclampsia has developed in some near-term pregnant females, with or without ENU treatment, providing a useful animal model for toxemia of pregenancy in man.

Significance to Biomedical Research and the Program of the Institute: With the recognition of the association between exposure to diethylstilbestrol *in utero* and development of vaginal adenocarcinoma during the second decade of life, the possible significance to human health of transplacental chemical carcinogens has become increasingly a matter of concern. Experimental studies on transplacental chemical carcinogenesis have been limited to rodent species, which appear to differ significantly from man in ways likely to be of importance for understanding the different consequences of exposure to chemical carcinogens during fetal versus adult life. Among these differences are the more rapid rates of fetal and neonatal development and maturation in rodents. The short latencies for tumor appearance and the type of tumors observed to date in the patas monkey provide experimental support for the position that at least some tumors of infancy and childhood, including certain congenital tumors, may result from prenatal exposure to carcinogens.

Proposed Course: Particular emphasis will be placed on defining the periods of maximal sensitivity to ENU in different organ systems of the fetus. Further studies on the acute toxicity and subsequent DNA repair as they relate to carcinogenesis will be initiated. The possible enhanced susceptibility of pregnant females to carcinogens, due to risk of placental neoplams, will also be investigated. The comparative response to diethylnitrosamine, a carcinogen requiring metabolic activation, will be investigated.

Date Contract Initiated: October 23, 1974.

Current Annual Level: $222,248

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CP-43366)

Title: Analysis of Regulatory Control of Cell Proliferation in Normal, Premalignant and Malignant Colonic Tissues in Familial Polyposis

Contractor's Project Director: Dr. Martin Lipkin

Project Officer (NCI): Dr. Carl E. Smith

Objectives: To define the phenotypic stages of preneoplasia in cells of humans at increased risk for colon cancer, and to develop well characterized human cells and population groups for studies of the mechanisms of carcinogenesis and its prevention in man.

Major Findings: 1) Development of Population Registries -- New population registries of individuals who are at increased risk for colorectal cancer, and who have been patients at Memorial Hospital have been developed for use in current studies. These registries are now available to facilitate callup of subjects for the various studies underway. Diseases recorded include familial polyposis, familial colon cancer, primary colon cancer, and multiple cancers with colonic plus breast and endometrial neoplasms in highest frequencies. Using the Kolmogorov-Smirnof 2-sample test, the ages on onset of colorectal cancer in population groups having colonic plus other cancers, familial colon cancer, and familiar polyposis were found to occur significantly earlier than in the general populations of the United States and Japan. Subjects developing neoplasia and familial aggregates of these various high risk population groups are under study, for identification of early phenotypic abnormalities associated with increased risk for large bowel neoplasia.

2) Abnormal Proliferation of Colonic Cells in Primary Colerectal Cancer -- After in vitro incorporation of tritiated thymidine ([^{3}H]TdR), a microautoradiographic analysis of the number of position of labeled colonic epithelial cell nuclei was carried out. Histologically normal colorectal mucosa was studied from 26 patients of whom half had colorectal cancer and half were controls. A highly significant upward shift of the proliferative cell compartment was observed in the cancer group; DNA synthesis predominated in the middle, or middle and upper thirds of the crypts of Lieberkuhn rather than in the lower third as observed in controls. The detection of this patchy alteration of epithelial cell renewal, frequently accompanying primary colorectal cancer, can provide a useful discriminatory parameter in studying relative risk of this population group.

3) Cytoskeletal Structure of Skin Fibroblasts in Familial Polyposis -- In the cytoplasm of well spread cultured normal fibroblasts, actin is organized into a network of cables that run the length of the cell just inside the adherent cell membrane. A disorganization of actin occurs in fibroblasts that have become tumorigenic as a result of oncogenic transformation. A disruption in actin organization was found in cultured skin fibroblasts obtained by biopsy from individuals with familial polyposis. Findings indicated that immunofluorescent localization of actin within cultured skin fibroblasts may be useful in the early identification of affected individuals.

4) Colonic Surface Epithelial Cells Obtained by Pulsatile Lavage -- Surface epithelial cells were obtained by pulsatile lavage of colonic mucosa, and incubated with [^{3}H]TdR for microautoradiographic observations. The presence of surface labeled epithelial cell nuclei derived from flat

mucosa was observed in 62% of 21 patients who currently had colon cancer or adenomas. Comparative observations also were made using the lavage technique in mice injected with 1,2-dimethylhydrazine. Mice were shown to have [^{3}H]TdR labeled cells and cellular atypia while being hemoccult negative and asymptomatic for overt disease.

5) Sequence Specificity of Protein-RNA Interaction in Human Colon Carcinoma Cells -- Ten to 15 percent of pulse labeled nuclear RNA in these cells is protected from digestion by exogenous nuclease. The protected RNA can be recovered in a ribonucleoprotein structure which sediments at approximately 2S, and which consists of a fragment of protected RNA 26 nucleotides long, and 3 major peptides, two at 66,000 daltons and a predominant species at 44,000. It was demonstrated that the protected RNA is very rich in guanosine and cytidine, and represents a specific subset of all sequences in heterogenous nuclear RNA.

6) Ribonucleoprotein Structure of Nuclear RNA in Human Colon Carcinoma Cells -- The ribonucleoprotein structure of two classes of relatively stable nuclear RNA has been studied. The first class represents a heterogeneous population, whose molecular weight distribution is similar to that of pulse labeled RNA, but whose ribonucleoprotein structure differs in that the RNA is in structures of slightly higher buoyant density and less accessible to digestion by exogenous nuclease. The second class of molecules represents a set of low molecular weight (22K-76K) RNA species (lmwRNA), each having a discrete molecular weight. These species are highly stable and not efficiently labled by incorporation of radioactive precursor. However, since they are highly methylated they can be labeled with [^{3}H]methyl methionine. While the lmwRNA species are extensively nicked by exogenous nuclease, a very high percentage (50-100%) of the methylated nucleotides (representing methyl incorporated onto the base, 2'-O-ribose, and 5' cap structure) remain in fairly large, acid and alcohol precipitable oligonucleotides. These data suggest that methylated nucleotides may be preferential sites of RNA-protein interaction.

Significance to Biomedical Research and the Program of the Institute: These findings have identified phenotypic abnormalities that develop in human cells as they undergo neoplastic transformation. They have provided means now used to detect individuals with expressed or latent inherited polyposis, a disease leading to colon cancer. The results also provide well defined human cells that can be used in studies that attempt to identify carcinogens involved in the development of colon cancer, and the interaction of inherited and environmental elements. In addition, the results provide well defined human population groups that will be used in experiments designed to prevent the development of colon cancer in man..

Proposed Course: Studies will be continued to develop markers or indices defining the stages of preneoplasia in cells of these human population groups at high risk for colon cancer. Related studies will be carried out on the high risk population groups to identify abnormal constituents of fecal contents. Findings of abnormal phenotypic characteristics will be quantitated for the classification of individuals and population groups,

in order to initiate experiments designed to prevent the development of colon cancer. The progression of phenotypic abnormalities in these cells that may be induced by chemical carcinogens will be studied.

Date Contract Initiated: October 15, 1971

Current Annual Level: $220,619

MICROBIOLOGICAL ASSOCIATES, INC. (N01-CP-02199)

Title: Laboratory Service for Support in Carcinogenesis Bioassay and Related Activities

Contractor's Project Director: Dr. Martin L. Wenk

Project Officer (NCI): Dr. Jerry M. Rice

Objectives: This contract provides diversified laboratory support services for the Carcinogenesis Program of NCI. Experimental protocols developed in collaboration with NCI investigators, and authorized by an internal NCI Program Administration Committee, are carried out through the use of contract facilities for the following functions; 1) holding, treatment and observations (including gross pathological evaluations) of mice, hamsters, rats, guinea pigs, rabbits or other small laboratory animals; 2) maintenance of athymic (Nu/Nu) mouse colony for determination of oncogenic potential of cultured cells and tissues and for xenotransplantation of human and other foreign tissues for carcinogenesis studies; 3) collection, preservation and histological processing of tissues as specified by individual protocls; 4) application of biochemical procedures or in vitro culturing techniques to various model systems associated with carcinogenesis; 5) preservation and processing of material for the production of 1 micron sections for high resolution light microscopy or the production of epoxy-embedded material for preparation of ultra-thin electron microscopy sections at NCI; and 6) application of techniques for autoradiography employing cultured tissue, 5 micron paraffin or 1μ epoxy-embedded materials.

Current experimentation emphasizes the development of rodent animal models for the most common forms of human neoplasms, the utilization of animal models to define the pathogenesis of such neoplasms and the evaluation of parameters that modify the incidence or severity of chemically induced tumors.

Major Findings: During the past year, support has been made available to 19 NCI investigators in conducting 34 individual experimental protocols. Contract efforts were concentrated in the following areas: 1) Development of in vivo and in vitro assays for malignant transformation; 2) development of animal models for significant human tumors including pancreatic, perinatal and gastrointestinal tumors; 3) evaluations of the relationship

between chemical carcinogenesis and viral expression in mammary carcinogenesis; 4) utilization of various animal models for an evaluation of enhancing or inhibiting factors upon chemical carcinogenesis (factors include age, sex or strain determined susceptibility, co-carcinogenic compounds, anti-neoplastic compounds, dietary modifications and route of carcinogen exposure); 5) utilization of model systems for histogenesis studies involving several chemically induced tumors; and 6) the utilization of contract facilities for services in support of intramural NCI research programs including the holding and supply of treated or untreated animals as dictated by approved protocols.

Signifance to Biomedical Research and the Program of the Institute: This contract offers the ability to conduct long-term in vivo experimentation involving large animal populations, to conduct rapid tests of new approaches in experimental pathology related to chemical carcinogenesis, and the ability to follow-up rapidly on studies that are relevant to the overall objectives of the Carcinogenesis Program.

Proposed Course: To continue to offer a diversified facility for the support of collaborative progrms performed with scientific investigators from the Carcinogenesis Program of NCI.

Date Contract Initiated: June 4, 1970

Current Annual Level: $810,735

MIDDLESEX HOSPITAL MEDICAL SCHOOL (NO1-CP-75955)

Title: Studies of Carcinogenesis in Human Tissues.

Project Director: Dr. R. Marian Hicks

Project Officer (NCI): Dr. Curtis C. Harris

Objectives: 1) To obtain viable normal tissue, with intact urothelium supported by mesenchyme, from human bladders by transurethral biopsy and occasionally from fresh cystectomy specimens; 2) To establish long-term (2-4 months) in vitro organ cultures of normal human bladder explants and to establish cell cultures in parallel; 3) To determine the parameters for normal growth of normal human urothelium in vitro in organ culture by comparison with parameters already established for rat bladder explants in vitro and rat and human bladder in vivo; and 4) To conduct carcinogenesis studies using human bladder explants.

Major Findings: The contract was initiated in September, 1977. The first three months were occupied in adding proposed staff, buying supplies, extra incubators, and so forth. Actual experimental work started in January 1978 and has been under way for nearly three months. In that time 14 transurethral biopsies have been obtained of bladder with intact urothelium from patients without neoplastic disease, and 10 biopsies from apparently normal areas of tumor-bearing bladders.

Biopsies have been examined a) immediately after removal from the patient; b) after transportation to the laboratory in various media; and c) after short-term (2-14 days) culture in various media to determine the optimum conditions for sampling, handling and growth.

The surgeons have routinely used distilled water for irrigation of tumor-bearing bladders before biopsy. It has been found, however, that samples taken under water are not, in general, well preserved. The superficial cells of the urothelium slough and the lower layers are oedematous. By contrast, it appears that urothelim from patients irrigated with glycine solution instead of distilled water are much healthier in appearance.

Observation indicates that the urothelium remains healthy and viable when biopsies are immediately placed in a cold Waymouth's medium with HEPES buffer and are transported to the laboratory in this medium.

Healthy, normal-looking explants have been maintained for up to 14 days in Waymouth's medium MB 752/1 supplemented with ferrous sulphate, hydrocortisone sodium succinate and 10% newborn calf serum, with added penicillin and streptomycin. In this medium the urothelium remains healthy and there is no change in growth pattern. For comparison, a few papillary tumors have been cultured successfully in the same medium. Other media being currently investigated include Waymouth's medium without the various supplements and Ham's medium with and without supplements. The gas phase used throughout has been 5% carbon dioxide in air.

Significance to Biomedical Research and the Program of the Institute: These very early findings indicate that normal, viable, human bladder biopsies can be obtained and cultured in vitro. Only short culture periods have been attempted so far, but based on contractor's experience with the rat bladder, it is not anticipated that there will be difficulty with long-term cultures. The viability of the project is thus confirmed.

Proposed Course: The viability and growth of cultures in different media will be assessed by histology and then by transmission and scanning electron microscopy. The length of culture will be extended in the optimum medium from 14 days to 4 months, and normal bladders in culture will be compared with bladder tumors in culture for the same period of time.

Date Contract Initiated: September 30, 1977

Current Annual Level: $83,144

MIDDLESEX HOSPITAL MEDICAL SCHOOL (N01-CP-75938)

Title: Long-Term Studies of Prevention of Epithelial Cancer by Retinoids.

Contractor's Project Director: Dr. R. Marian Hicks

Project Officer (NCI): Dr. Morton H. Levitt
Dr. Carl E. Smith
Dr. Michael B. Sporn

Objectives: The objective of this program is to determine the relative effectiveness of different retinoids in preventing and/or reversing neoplastic growth of the epithelium lining the urinary bladder of carcinogen treated rats. This will be assessed both by long-term studies in which the life-time response of carcinogen treated animals to retinoids is examined, and also by careful histological and electron microscopical assessment of the subcellular changes occurring in the urothelium of carcinogen and/or retinoid-treated animals. Any relative incidental toxicities of the retinoids will be assessed histologically.

The program was initiated September, 1977. All the staff required to work on the project has been appointed and a supply of inbred Fischer F344 rats obtained.

Approximately 250 rats have received a full carcinogenic treatment with the selective bladder carcinogen hydroxybutylbutanol nitrosamine (OHBBN) and these and control untreated animals are now being maintained either on a 13-cis-retinoic acid containing diet or a placebo diet. These animals are being sequentially sampled for histological and ultrastructural tissue assessment. Other groups (altogether a further 350 animals) are currently receiving carcinogen treatment and these animals will be maintained on different concentrations of another retinoid (retinoic acid ethylamide) for a life-time assessment of their response.

Major Findings: It is too soon to observe the effect of 13-cis-retinoic acid on the neoplastic response of the eurothelium to OHBBN since the retinoid has only been administered for 4 weeks so far. The growth curves of the animals indicate the dose of retinoid used is well tolerated. The initial response of the urothelium to the OHBBN indicates that there has been preneoplastic transformation of the urothelium of all animals examined so far.

Significance to Biomedical Research and the Program of the Institute: The results obtained so far confirm the viability of the project and show the model in use has the potential to produce the information required.

Proposed Course: To continue the investigations as indicated above.

Date Contract Initiated: September 30, 1977

Current Annual Level: $84,000 (Supported by Carcinogenesis Testing Program)

MIDWEST RESEARCH INSTITUTE (N01-CP-75911)

Title: Synthesis of Radioactive Retinoids for Metabolic and Pharmacologic Studies Relating to Prevention of Lung Cancer and Other Epithelial Cancers.

Contractor's Project Director: Dr. Hans H. Kaegi

Project Officers (NCI): Dr. Charles Frolik
Dr. Anita Roberts

Objectives: The objective of this program is the synthesis, isolation, purification, analysis, and supply of specified quantities of NCI-selected radioactive retinoids for use as tracers in metabolic or pharmacologic studies, both in vivo and in vitro. The program involves the preparation of different retinoids with possible modification of the ring, side chain, or terminus and variations in both the radioactive isotope (^{14}C or ^{3}H) and the position of label incorporation.

Major Findings: A synthetic scheme has been worked out for the preparation of the first compound, 12-^{3}H-cis-retinoic acid and the hot preparation will be started presently. Cold runs for the other two compounds, 13,14-$^{14}C_2$-all-trans-retinoic acid and 14-^{3}H-all-trans-retinoic acid, are in progress.

Significance to Biomedical Research and the Program of the Institute: In connection with the ongoing collaborative program relating to prevention of epithelial cancers by the use of retinoids (Vitamin A and its synthetic analogues) the need for radioactively labeled compounds of this class has become imperative. Without the aid of radioactive tracers the investigation of physiological or pharmacological mechanisms, the metabolic rate and pharmacokinetic properties and more would be very tedious if not impossible.

Proposed Course: Tritium and carbon-14 labeled compounds will continue to be synthesized upon the request of NCI.

Date Contract Initiated: September 30, 1977

Current Annual Level: $189,828

MINNESOTA, UNIVERSITY OF (N01-CP-55702)

Title: Rhythmometry on Japanese and North-American Female Volunteers of Different Age Groups.

Contractor's Project Director: Dr. Franz Halberg

Project Officer (NCI): Dr. D. Jane Taylor

MIDWEST RESEARCH INSTITUTE (NO1-CP-75911)

Title: Synthesis of Radioactive Retinoids for Metabolic and Pharmacologic Studies Relating to Prevention of Lung Cancer and Other Epithelial Cancers.

Contractor's Project Director: Dr. Hans H. Kaegi

Project Officers (NCI): Dr. Charles Frolik
Dr. Anita Roberts

Objectives: The objective of this program is the synthesis, isolation, purification, analysis, and supply of specified quantities of NCI-selected radioactive retinoids for use as tracers in metabolic or pharmacologic studies, both in vivo and in vitro. The program involves the preparation of different retinoids with possible modification of the ring, side chain, or terminus and variations in both the radioactive isotope (^{14}C or ^{3}H) and the position of label incorporation.

Major Findings: A synthetic scheme has been worked out for the preparation of the first compound, 12-^{3}H-cis-retinoic acid and the hot preparation will be started presently. Cold runs for the other two compounds, 13,14-$^{14}C_2$-all-trans-retinoic acid and 14-^{3}H-all-trans-retinoic acid, are in progress.

Significance to Biomedical Research and the Program of the Institute: In connection with the ongoing collaborative program relating to prevention of epithelial cancers by the use of retinoids (Vitamin A and its synthetic analogues) the need for radioactively labeled compounds of this class has become imperative. Without the aid of radioactive tracers the investigation of physiological or pharmacological mechanism, the metabolic fate and pharmacokinetic properties and more would be very tedious if not impossible.

Proposed Course: Tritium and carbon-14 labeled compounds will continue to by synthesized upon the request of NCI.

Date Contract Initiated: September 30, 1977

Current Annual Level: $189,828

MINNESOTA, UNIVERSITY OF (NO1-CP-55702)

Title: Rhythmometry on Japanese and North-American Female Volunteers of Different Age Groups.

Contractor's Project Director: Dr. Franz Halberg

Project Officer (NCI): Dr. D. Jane Taylor

Objectives: Sampling schedules are to be explored to determine whether or not women with different risk for breast cancer exhibit differences in hormonal and/or other physiologic patterns, notably but not exclusively differences in rhythmic changes with frequencies ranging for selected variables from one cycle in a few minutes to one cycle in a year.

Major Findings: Plasma cortisol and prolactin were analyzed in samples obtained every 20 mintues for 24 hours in winter and again in spring and were found to exhibit a statistically significant circadian rhythm in most subjects.

A multivariate analysis based on 3 circadian rhythm characteristics (mesor, amplitude and acrophase) was applied first for plasma prolactin from the winter and spring quarters to find a statistically-significant difference between Japanese and Minnesotans, irrespective of age (P = .01). Separate tests for each characteristic suggest that differences in amplitude (Japanese greater than American) contribute most to the overall difference, although the circadian mean (mesor) also tends to be higher in the Japanese subjects. To pursue the question whether these observed differences may be due to the particular subjects involved, or to their wide age range, groups of 20-year-old women in Fukuoka City, Japan, and Minneapolis, Minnesota, gave blood at 7 consecutive 4-hour intervals during March 1978. (This study was added over and above the work negotiated with the National Cancer Institute -- with no cost for chemistries or numerical analysis.) Results on a small number of series thus far analyzed (9 Japanese, 3 Minnesotans) confirms the higher mesor in the Japanese (22.17 ng/ml versus 16.63 ng/ml in the Minnesotans) and the much higher average peak concentration and a lower trough concentration of plasma prolactin in Japanese as compared to North American subjects (46.83 $\pm$ 9.11 and 10.14 $\pm$ 4.87 versus 24.47 $\pm$ 4.14 and 13.03 $\pm$ 2.31). Since results on this sample agree with the somewhat higher mesor and the much higher amplitude in the Japanese women, found in studies supported by this contract with more extensive sampling, it seems likely that the judgment sample originally obtained will allow tentative yet highly suggestive inferences to the populations involved in Japan and Minnesota.

Multivariate comparison of rhythm characteristics for thyroxine between Japanese and Minnesotans does not show any statistically significant intergroup difference. A statistically significant difference in the ratio of estriol to the sum of estrone and estradiol was found between Japanese and North American women in the winter samples of 19 Minnesotans and 11 Japanese but not in the spring samples of 6 Minnesotans and 7 Japanese analyzed thus far. Whether ratios in Minnesotans but not in Japanese undergo a circannual rhythm remains to be investigated.

Significance to Biomedical Research and the Program of the Institute: The vast majority of health research and practice often directly involves ubiquitous rhythms. Accordingly, the results from this contract bear upon all research supported or guided by the National Cancer Institute, whenever rhythmic variables are involved. This study will provide basic data

on hormonal levels in women of various age groups and physiological methodologies of importance to future carcinogenic research in humans.

Proposed Course: As more results of chemical determinations on already collected samples become available, they have to be evaluated with several kinds of analysis-batteries, including: 1) the use of conventional methods; 2) special methods already developed and programmed; and 3) special methods as yet to be developed or adapted and, in any event, programmed.

In summary, this project's data base has reached a new dimension, not only by the consideration of the large amount of chemical and biophysical analyses completed (hormonal analysis on blood sampled every 20 minutes during 24 hours, 4 times a year), but also in view of the many other physiologic variables examined during each hospitalization (blood pressure every 10 minutes, breast surface temperature, activity and EKG, continuously recorded for 24 hours) and from self-measurements during one month covering hospitalization) (urine, blood pressure, oral, axillary and breast surface temperature). Analyses also are becoming more complex since we want to investigate many frequencies and their interactions with each other, not only for each individual but also for comparisons among different age, risk and geographical location groups.

Date Contract Initiated: June 30, 1975

Current Annual Level: $160,000

MINNESOTA, UNIVERSITY OF (NO1-CP-33364)

Title: Polycyclic Hydrocarbon Metabolism in the Respiratory Tract

Contractor's Project Director: Dr. Lee W. Wattenberg

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The objectives are 1) to determine the mechanism whereby phenolic antioxidants inhibit neoplasia of the lung resulting from administration of carcinogens; and 2) to ascertain structural requirements for compounds to act as inhibitors.

Major Findings: During the past year studies of butylated hydroxyanisole (BHA) under conditions in which it inhibits pulmonary neoplasia due to benzo(a)pyrene (BP) have shown that this antioxidant alters the microsomal metabolism of the carcinogen so as to increase its detoxification. An improved high resolution high pressure liquid chromatographic technique has been developed for the study of microsomal metabolism of BP. Several striking findings have been obtained employing liver microsomes from mice which had been fed BHA. There is almost a total loss in the formation of BP-4,5-oxide and 9OH-BP. In contrast, an increase in 3OH-BP occurs. In addition to metabolite studies, investigation of the microsomal metabolism of BP to metabolites binding to DNA have been carried out. Previously, it

was demonstrated that microsomes from animals which had received BHA show a reduced metabolism of BP to metabolites binding to DNA. These studies have been extended using as the substrate BP-7,8-dihydrodiol. With this substrate as with BP, BHA causes a reduction in the amount of binding of metabolites to DNA. Since 7,8-dihydrodiol has now been shown to be a proximate carcinogen, inhibition of the step involving its conversion to a metabolite binding to DNA presumably BP-7,8-dihydrodiol-9,10-oxide, would appear to be of considerable importance.

In other work, the effects of BHA on the concentration of BP and its metabolites in blood and tissues has been determined. If BHA is administered four hours prior to challenge with ^{3}H-BP, there is no alternation of the total amount of BP and its metabolites in whole blood, stomach, small intestine, liver or lung. However if DNA is isolated, it is found that the DNA of lung has approximately 60% as much bound BP as the DNA from the lung of control mice.

Studies are in progress relating chemical structure of antioxodants to their capacity to inhibit pulmonary neoplasia in A/HeJ mice. Thus far seven antioxidants have been studied. Their inhibitory effects are as follows: (ratio of pulmonary adenomas in mice receiving the test compound to pulmonary adenomas in control mice) BHA, 0.40; 2,6-di-t-butylphenol, 0.43; 2-t-butyl-4-thiomethoxyphenol, 0.48; 2-t-butyl-octyloxyphenol, 0.53; 2-t-butylphenol, 0.56; 4-hydroxyanisole, 0.64; 2-t-butylhydroquinone, 0.94. Work has been initiated to develop an in vitro test for predicting the inhibitory capacities of antioxidants on carcinogen-induced pulmonary neoplasia. A modification of the Ames test has been employed using microsomes from control animals and from mice which have received BHA. The microsomes from mice which have received BHA produce less mutagenic metabolites of BP than microsomes from corresponding controls.

Significance to Biomedical Research and the Program of the Institute: A knowledge of the mechanism of inhibition of carcinogen-induced pulmonary neoplasia could be of value in assessing the effects of environmental factors on susceptibility to neoplasia. It also would serve as a basis for the development of highly potent compounds which could be used as inhibitors in selective population groups at high risk.

Proposed Course: The course as outlined in the original contract will be followed.

Date Contract Initiated: June 23, 1973

Current Annual Level: $136,906

NEBRASKA, UNIVERSITY OF (at Lincoln) (N01-CP-33289)

Title: Chemical Carcinogen-Induced Noduligenesis and Tumorigenesis in Whole Mouse Mammary Gland Organ Culture.

Contractor's Project Officer: Dr. Mihir R. Banerjee

Project Officer: Dr. Carl E. Smith

Objectives: While malignant transformation, caused by oncogenic chemicals is a reality in cell culture, successful "transformation" of epithelial cells in an organ culture yet remains a challenge. Accordingly, the following studies were undertaken. 1) To assess the influence of less oncogenic hydrocarbon compounds on induction of nodule-like alveolar lesions (NLAL) in the mammary gland in organ culture; 2) To assess whether the mammary glands in organ culture exhibit biochemical characteristics such as carcinogen binding to DNA and induction of the metabolizing enzyme(s), arylhydrocarbon hydroxylases (AHH) after DMBA treatment; and 3) To assess biological characteristics of DMBA-induced NLAL and/or other latent transformed cells by mammary fat-pad transplantation.

Major Findings: The whole 2nd thoracic mammary gland of 3-4 week old female BALB/c mouse is induced to pregnancy-like alveolar development after 9 days of cultivation in a chemically defined culture medium containing the hormones insulin (I), prolactin (Prl), aldosterone (A) and cortisol (F). Treatment of the gland with DMBA (2 μg/ml) for a 24-hour period between 3 and 4 days of culture induces NLAL in 80% of the gland and these lesions become visible when normal alveoli are allowed to regress in medium with I + A. Consistent with their known characteristics equimolor concentrations of less potent hydrocarbon, dibenzanthracene (DBA), benzanthracene (BA) and anthracene (A) under similar culture conditions exhibit markedly reduced NLAL-inducing action. Biochemical studies revealed that ^{3}H-DMBA (or ^{14}C-DMBA) binds to purified DNA of the mammary cells in culture, and spectral analysis of DNA indicated the presence of DMBA in mammary DNA, as measured by absorbance at 310 nm suggesting a covalent binding of the hydrocarbon. DMBA was found to induce a fivefold increase in AHH activity of the mammary gland in culture. Thus the highly potent oncogenic compound, DMBA, undergoes metabolic activation in a typical manner in the present culture system.

Significance to Biomedical Research and the Program of the Institute: The findings thus far appear encouraging with regard to potential of the present organ culture system to develop into a bioassay for monitoring the action of oncogenic chemicals in breast carcinogenesis.

Proposed Course: Assessment of potential tumorigenicity of DMBA-induced NLAL and/or latent transformed mammary cells are underway by mammary fat-pad transplantation in vivo. To date, out of nearly 100 animals carrying explants of DMBA treated (either as tissue fragments of dissociated epithlial cells) glands a few have shown some abnormal ductal and/or lobular alveolar outgrowths. The irreversible nature of these abnormalities is being assessed by serial fat-pad transplantation. Animals carrying different types of explants are also being maintained on a long term basis for possible tumor incidence. Occurrence of anticipated mammary tumors in the outgrowths derived from the explants may take 8-12

months from the date of transplantation. The frequency of tumor incidence in DMBA-induced nodule outgrowths in studies in vivo is known to be very low (6%).

Date Contract Initiated: June 1, 1973

Current Annual Level: 0

NEW ENGLAND NUCLEAR CORPORATION (N01-CP-75937)

Title: Synthesis of Radioactive Retinoids for Metabolic and Pharmacological Studies Relating to the Prevention of Lung Cancer and Other Epithelial Cancers

Contractor's Project Director: Dr. David Ahern

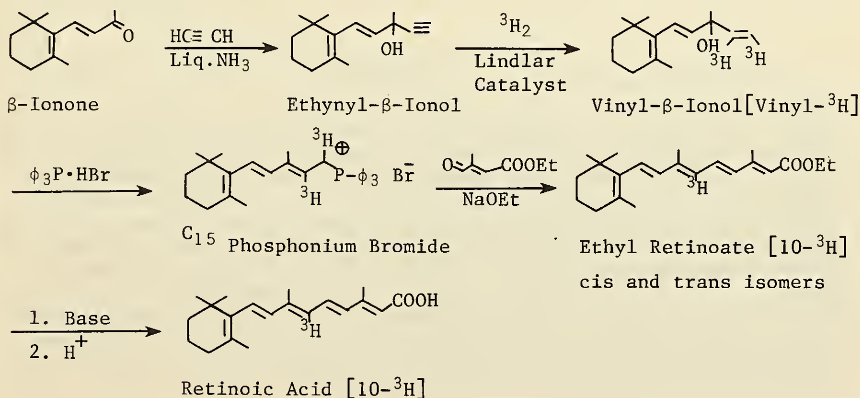
Project Officer(s) NCI): Dr. Charles Frolik
Dr. Anita Roberts

Objectives: Synthesis of specifically labelled retinoids as directed by NCI

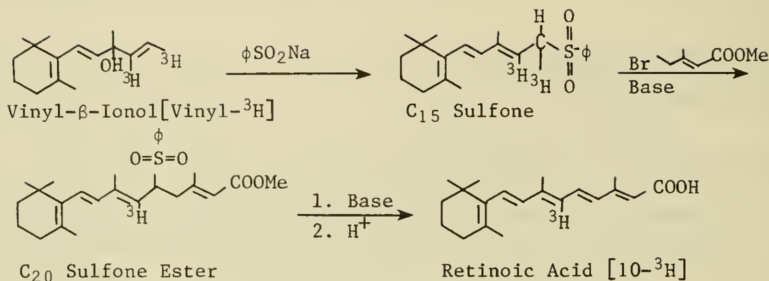
Major Findings: Several approaches to the synthesis of retinoic acid [10-^{3}H] were evaluated as to time, labor, availability of precursors, and ease of selective labelling with tritium.

Two methods which diverged from a common precursor, β-vinyl-ionol [vinyl-^{3}H] were most promising and chosen for evaluation as is indicated in Schemes I & II.

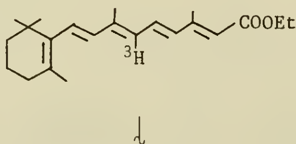
Scheme I



Scheme II



Scheme I indicates a classical industrial process. It involves the ethynylation of β-ionone, catalytic reduction with tritium to the olefin, conversion to a phosphorane, and a Wittig reaction with β-formylethylcrotonate. Each of these reactions proceeds in good yield and tritium is introduced at the catalytic reduction stage. Catalytic reduction with tritium introduced the isotope at both the C-10 and C-11 positions, but once the phosphorane is formed, ^{3}H at the C-11 position becomes exchangeable. The phosphorane was condensed with ethyl β formyl crotonate in basic media, which also exchanged out ^{3}H on C-11 position. The resulting mixture of isomers of ethyl retinoate ^{3}H were separated by HPLC. Tritium NMR of the purified ethyl retinoate ^{3}H indicated a single ^{3}H resonance when H decoupled and a doublet when H coupled. This is in good agreement for structure ⫰



⫰

Scheme II indicates a synthesis of all trans retinoic acid [10-^{3}H] through sulfone intermediates. Starting from the common intermediate vinyl-β-ionol [vinyl-^{3}H], the sulfone was prepared and purified. Tritium NMR of this compound confirmed that the two positions α and β to the sulfone were labelled. Basic exchange removed the tritium α to the sulfone, which was shown to be quantitative by tritium NMR. This sulfone was condensed with trans-β-bromo-methyl crotonate, to give after elimination all trans retinoic acid [10-^{3}H]. At the present time difficultly in purifying the [10-^{3}H] retinoic acid is being encountered. Investigators are exploring whether this is due to radiodecomposition or isomerization due to the inherent radioactivity in the molecule.

Significance to Biomedical Research and the Program of the Institute: Radiolabelled compounds are almost indispensable in studies of metabolic pathways and pharmacologic disposition.

Proposed Course: Continue synthesis of labelled retinoids.

Date Contract Initiated: September 30, 1977

Current Annual Level: $80,242

NEW YORK MEDICAL COLLEGE (NO1-CP-75949)

Title: Studies of Meabolic Capacity of Intestional Mucosa

Contractor's Project Director: Dr. Herbert S. Rosenkranz

Project Officer (NCI): Dr. Carl E. Smith

Objectives: 1) Investigate the capacity of the colonic mucosa to biotransform representative colon specific carcinogens and other substances to mutagenic and DNA-modifying substances; 2) Determine capacity of microsomal extracts of colonic mucosa to biotransform compounds to mutagenic and DNA-modifying substances; 3) Determine metabolic capacity of cell-free extracts derived from representative anaerobes to biotransform representative colon specific carcinogens and other compounds to mutagenic and/or DNA-modifying substances; and 4) Determine similarly the metabolic capacity of mixtures composed of colonic mucosa and anaerobes to biotransform compounds.

Major Findings: The initial period of this contract has been devoted to characterizing the optimal requirements of metabolic activation mixtures derived from representative anaerobes and to define optimal procedures for preparing microsomal extracts from intestinal mucosa. These goals have now been achieved.

Significance to Biomedical Research and the Program of the Institute: A number of colon-specific carcinogens of rats have been identified; some of these obviously require metabolic activation to their ultimate-acting form. Presumably the activation of these substances occurs in the colon and is mediated by the colon mucosa or the resident bacterial (mainly anaerobic) flora or by both. The present study is concerned with the ability of the colon mucosa and of the bacterial flora to activate potential colon-specific carcinogens to mutagens and substances endowed with DNA-modifying activity. An understanding of these mechanisms will permit conclusions concerning the role of diet and intestinal flora in the induction of colon cancer.

Proposed Course: To investigate the ability of microsomal preparations from intestinal mucosa and cell-free extracts from anaerobic bacteria to activate colon-specific carcinogens as well as presumptive carcinogens to

metabolites possessing mutagenic and/or DNA-modifying activity and to determine the chemical nature of these metabolites.

Date Contract Initiated: September 30, 1977

Current Annual Level: $84,016

NEW YORK UNIVERSITY (NO1-CP-33260)

Title: Studies in Pulmonary Carcinogenesis

Contractor's Project Director: Dr. Roy E. Albert

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The objectives of the contract are threefold: to develop animal models and techniques for the study of inhalation carcinogenesis as it relates to humans; to use these models and techniques to test and evaluate the carcinogenic potential of industrial and environmental pollutants; and, to examine the mechanisms of lung carcinogenesis and the progression of malignant changes in the respiratory tract. Human lung cancer is often multi-factoral in nature, resulting from long term, low level exposures to agents in combination. This laboratory has pioneered in the development of animal models to study the effects of combined exposures.

Major Findings: A variety of techniques have been developed or utilized in this laboratory to duplicate bronchogenic carcinoma in rats and hamsters including: thread transfixion in the mouse, intrabronchial pellet implantation in the rat, direct intrabronchial instillation in the hamster and inhalation with rats and hamsters.

Dose-response relationships have been shown with the pellet implant technique in rats using polycyclic hydrocarbons. An extensive study with hamsters using intratracheal instillations of methylcholanthrine showed excellent dose-response relationships. Data from the serially-sacrificed groups helped to define the progression of preneoplastic changes.

The effects of combined exposure to air pollutants have been studied extensively in this program. This laboratory was the first to produce lung cancer in rats through combined inhalation exposures to benzo(a)-pyrene and sulfur dioxide. Rats exposed to the carcinogen and sulfur dioxide simultaneously developed significantly higher numbers of cancers than groups given identical exposures consecutively.

Studies with benzo(a)pyrene and nitrogen dioxide (a component of air pollution and cigarette smoke), showed some enhancement in cancer induction by the combined exposure compared to the carcinogen alone, although the effect was not as strong as with benzo(a)pyrene and sulfur dioxide.

To further investigate this phenomenon and develop another model for combined carcinogenesis, the hamster intratracheal technique was combined with inhalation exposures to sulfur dioxide. Benzo(a)pyrene, alone, at doses which were negative for tumor induction, produced both squamous cell and adenocarcinomas when combined with chronic exposure to SO_2.

By use of the pellet technique, calcium chromate was identified for the first time in this laboratory as the agent most likely responsible for the lung cancer seen in the chromium industry. In addition, chronic inhalation of calcium chromate and a process residue containing calcium chromate by rats and hamsters produced carcinomas in both species.

Polyurethane foam dust and a reinforced polyester-fiberglass plant dust were used in intubation and short term inhalation studies. Polyester-fiberglass and polyurethane dust produced centrilobular emphysema and polyurethane produced a suggestive carcinogenic response.

Two chlorinated ethers, widely used industrial alkylating agents, were tested by inhalation. Both chloromethyl methyl ether and bis (chloromethyl)ether were carcinogenic. The bis compound, moreover, was extremely carcinogenic at very low levels (0.1 ppm for 80 exposures). As a result of these studies, both compounds are now listed by OSHA as carcinogens and an industry-wide epidemiology study by this Institute has demonstrated the induction of human cancer in workers exposed to bis(chloromethyl)ether.

Preliminary studies in this laboratory and elsewhere have shown that hydrochloric acid and formaldehyde can combine to form bis(chloromethyl)-ether. A chronic exposure study to both these agents in combination is underway. A number of the exposed rats in this study have developed squamous cell carcinoma of the nasal cavity, suggesting a new and possibly serious industrial cancer hazard.

Studies with rats and hamsters are being done with another alkylating agent, dimethycarbamoyl chloride, an industrial intermediate used in pesticide and drug synthesis. This agent is showing strong carcinogenicity for both rats and hamsters. Ninety-four of 98 rats developed squamous cell carcinoma of the nasal cavity with all animals dead by 52 weeks. This tumor is also appearing in rats now being chronically exposed at 0.3 ppm but with longer induction times. Hamsters, currently undergoing exposure at 1 ppm, are also developing squamous cell carcinoma of the nasal cavity. This incidence,however, is much lower than seen with rats at this level (25/60 to date) and appears much later. Regulating agencies, industry in the United States and overseas, and the scientific community have been informed of these results and will be made aware of future developments as these studies progress.

Similarly, epichlorohydrin, another widely used industrial intermediate, is currently under test in rats and is showing carcinogenic effects in the respiratory tract. These preliminary results have also been made available to the federal regulating agencies, industry and the scientific community.

Studies are being initiated in an attempt to develop an animal model for arsenic, which is a human carcinogen. Under other support, an animal model for benzene carcinogenesis is being developed, with encouraging results in mice already obtained for this human carcinogen.

Significance to Biomedical Research and the Program of the Institute: Work carried out under the contract has led to three highly significant broad achievements. First, procedures and systems have been developed which permit the accurately controlled exposure of experimental animals to potentially carcinogenic materials. Secondly, through the use of these procedures, a number of specific substances have been identified which are likely to pose a hazard to the human population. As a result, steps have been taken to limit worker exposure to these agents. And finally, these studies have highlighted the importance of interactions between inhaled mixture components, the enhancement of benzo(a)pyrene carcinogenicity by SO_2 being a prime example.

These contributions to understanding the factors influencing respiratory carcinogenesis have been and will continue to be in the forefront of efforts to reduce the devastating toll taken by respiratory cancer in man.

Proposed Course: Above studies will continue in this contract as planned until the scheduled termination date December 31, 1978.

Date Contract Initiated: January 1, 1973

Current Annual Level: $395,975

NORTH CAROLINA, UNIVERSITY OF (NO1-CP-75956)

Title: Studies of Carcinogenesis in Human Tissue

Contractor's Project Directors: Dr. David G. Kaufman
Dr. Guy Photopulos

Project Officer (NCI): Dr. Curtis C. Harris

Objectives: The overall objective of this project is to determine whether specific insights into the causation of endometrial carcinoma can be derived by direct study of effects of chemical carcinogens on human endometrial tissue. The specific efforts which were scheduled to be undertaken during the first year of the contract included: Task I, relating to the obtainment of human tissue, and Task II, concerning the maintenance of human tissues in vitro.

Major Findings: Since obtainment of human tissues underlies all efforts of the program, these activities designated as Task I were the issues pursued to the greatest degree of completeness during the first six months of the contract. During this initial time period, procedures have been

put into practice for obtainment of human endometrial tissue, largely as were described in the proposal. An essential feature of the procedures for obtaining appropriate human endometrial tissue has rested upon the close interaction between the co-principal investigator and the chief resident on the Gynecology Service. They have made a practice of meeting each week to evaluate the patients scheduled for hysterectomies the following week. The patients are considered in terms of the indications for the surgery and their age, so as to minimize the likelihood for intrinsic endometrial disease in the hysterectomy specimens. This procedure has worked well for the obtainment of desirable experimental samples, and, more importantly, it has provided an additional means of limiting the risk of compromise of tissue needed for pathologic evaluation in case of significant intrinsic endometrial disease.

The second major area of effort during this first six month period related to Task II, which concerns the development of methods for the maintenance of human tissues in vitro. The principal emphasis of the contract is to develop methods for maintaining organ cultures of endometrium, but concurrently methods are being evaluated for establishing and maintaining cell cultures. A variety of techniques for cell and organ culture establishment and several types of tissue culture media have been used in this first period of the contract. With few exceptions, the results have been extremely promising to the present. Organ cultures have been maintained in a variety of tissue culture media including one which is chemically defined (without supplementation by serum). To the present time, CMRL-1066 supplemented with 10% fetal calf serum has best preserved the organ cultures. Some organ cultures have been successfully maintained for nearly four months. Under these conditions, epithelial morphology has been well preserved. At the same time, primary cell cultures have been established from all specimens with which this technique was attempted. Again CMRL-1066 medium supplemented with 10% fetal calf serum appears to be the most favorable medium for growth of these cultures. Colonies of a variety of morphologic types have been identified in these cultures which, though not yet conclusively identified, bear a strong suggestive relationship to a variety of the intrinsic endometrial cell populations seen in the intact tissue. It has been determined that plates can be split and subcultured, and this technique has been applied to the subculturing of colonies of specific morphologic types. Initial efforts have demonstrated qualitative response to supplementation of the media with estrogen and preliminary studies have suggested that appropriate combinations of estrogen and progesterone cause the production of PAS-stainable materials within these cells.

Significance to Biomedical Research and the Program of the Institute: The overall objective of this program is to transform human endometrial tissue in vitro with chemical carcinogens. These studies may contribute to an understanding of the causation of human endometrial cancer and help explain interindividual variations in susceptibility to this disease. The accomplishments to date in the performance of this contract represent the first steps in efforts to achieve these objectives.

Proposed Course: During the next year quantitative data will be accumulated to determine the optimal conditions for maintenance of organotypic and cell cultures of human endometrium. Further studies will characterize the normal biologic properties of the cultured endomentrial tissue in comparison to the fresh tissue. Studies may also be initiated to determine the effects of carcinogens on the biologic properties of endometrial cultures.

Date Contract Initiated: September 30, 1977

Current Annual Level: $109,528

NORTHWESTERN UNIVERSITY (NO1-CP-75876)

Title: Histogenesis of Guinea Pig Pancreatic Adenocarcinoma

Contractor's Project Officer: Dr. Janardan K. Reddy

Project Officer (NCI): Dr. Carl E. Smith

Objectives: This project was initiated to confirm earlier reports of induction of pancreatic adenocarcinoma in guinea pigs by methylnitrosourea (MNU) and to delineate the histogenesis of pancreatic tumors. An additional objective was to manipulate this model so as to reduce the induction period and to increase the incidence of pancreatic tumors.

Major Findings: Several agents capable of inducing acute and chronic alterations in exocrine pancreas of the guinea pig were identified. These include, lasiocarpine, 4-hydroxyaminoquinoline-1-oxide(4-HAQO), cobaltous chloride, aflatoxin B_1, and azaserine. MNU also caused severe atrophy of the acinar tissue and fatty vacuolation in the acinar cell cytoplasm. The ultrastructural changes observed in acute and subacute injury were characterized by alterations in rough endoplasmic reticulum and zymogen granules. These changes are considered non-specific, although severe in intensity.

Two models of pancreatic regeneration were developed. Significant regenerative capacity was exhibited by pancreatic acinar cells following an experimental regimen of ethionine-protein free diet or after a single intravenous injection of 4-HAQO. It appears that the well differentiated acinar cell is capable of undergoing cell division following an appropriate stimulus.

A significant reduction in the latent period of pancreatic tumor development was noted in inbred strain 13 guinea pigs given MNU once weekly by gavage at a dose level of 10 mg/kg body weight. Histogenetic studies of these neoplasms revealed that exocrine acinar cells proliferate under the influence of the carcinogen and assume pseudoductular pattern. The presence of zymogen granules in cells lining these tubular areas strongly

suggests that MNU induced adenocarcinomas of the guinea pig are derived from transformation of acinar cells.

Although MNU-guinea pig model of pancreatic cancer proved to be a valuable system in clarifying the role of acinar cell in the genesis of pancreatic carcinoma, several deficiencies exist in this model which must be rectified before it can be successfully used for other studies. The high mortality rate among the animals given MNU during the early phases and the relatively low incidence of pancreatic tumors are the two major drawbacks.

Significance to Biomedical Research and the Program of the Institute: Because of the high incidence of pancreatic carcinoma in this country it is essential to develop suitable biological models of this cancer. Model development is one of the major goals and this project along with others is designed specifically to achieve this goal.

Proposed Course: This project was completed January 2, 1978. Data is being analyzed for final publication.

Data Contract Initiated: January 3, 1977

Current Annual Level: 0

ORGANIZATION FOR HEALTH RESEARCH (TNO) (NO1-CP-33330)

Title: Synergistic Interaction of Hormones and Neutron Irradiation on Mammary Gland Carcinogenesis

Contractor's Principal Investigator: Dr. Johannes J. Broerse

Project Officer (NCI): Dr. D. Jane Taylor

Objectives: To determine whether the synergistic interaction found with x-irradiation and estrogen in the rat mammary gland is reproduced with neutron irradiation and estrogen. There are three approaches to the objective: a) investigation of the nature of the dose effect relationships for X-rays and neutrons; b) study of the possible synergistic interaction of hormone administration and neutron irradiation; and c) determination of the RBE of different neutron beams to assess the relative risk of neutron irradiation. Three rat strains, Sprague-Dawley (SD), Wistar (WAG/Rij) and Brown Norway (BN) with expected different susceptibilities for the induction of mammary cancer have been irradiated with 300 kV X-rays and monoenergetic neutrons with energies of 0.5, 4 and 15 MeV. For each type of radiation and different dose levels, four subgroups are included: intact females, intact females treated with estrogen, hystero-ovariectomized females, and hystero-ovariectomized females with estrogen. The estrogen employed is Estradiol- 17β, (E_2) instead of diethylstilbestrol infused in pellets with a paraffin-cholesterol base.

Major Findings: A total of 5000 animals have been introduced into the study. Results are stored in a Data General Eclipse c/330 computer. Histological examinations of approximately 2500 animals have been completed and for this group the proportion of animals surviving without known tumor $P(t_k)$ has been calculated according to a life table analysis. From these results the cumulative tumor prevalence can be derived as $1-P(t_k)$. In the irradiated groups of intact females the first tumors generally appeared between 10 and 12 months after irradiation. For the nonirradiated control groups the latent period is considerably longer (e.g., 21.5 months in the Sprague Dawley). The cumulative prevalences for spontaneous tumor induction in the control groups of SD, WAG/Rij and BN rats at an age of 30 months are to 47, 26, and 12 percent, respectively. For the hystero-ovariectomized animals the tumor prevalence is considerably lower than for the intact control group. In the hormone-treated animals, both in the controls and in the irradiated groups, the tumors occur earlier than in the parallel groups of nonhormone-treated animals.

The majority of all mammary tumors observed in the rats were histologically classified as benign, the most frequent type being the fibroadenoma. Andenomas either of a lobular or intraductal papillary type, and fibromas were far less common. The most common types of carcinomas were simple adenocarcinomas or papillary adenocarcinomas or mixtures of the two. Spindle cell sarcomas of the mammary gland were extremely rare.

In order to derive dose-effect relationships from the life table curves, the cumulative tumor prevalence has to be scored at a time when the number of spontaneously occurring tumors is still very low. Cumulative mammary tumor prevalence has been determined for the three rat strains at an age of 22 months. From these dose-effect curves RBE values have been determined for the intact animals. For the 30 percent tumor prevalence level the RBE values for 15 MeV neutrons vary between 1.9 and 4.6 for the three rat strains. At the same level of prevalance an RBE of approximately 20 can be derived of the WAG/Rij rats after irradiation with 0.5 MeV neutrons (Broerse et al., 1978)

Significance to Biomedical Research and the Program of the Institute: The present program on radiation carcinogenesis is of high significance for the assessment of risks of ionizing radiation during occupational and accidental exposure. Furthermore, this type of research is of interest to allow a risk-benefit analysis of diagnostic procedures involving small doses of ionizing radiation. In this connection the topic mammography is presently under intensive discussion. The human data available for X- and gamma-radiation all pertain to large doses and show relatively large variations; consequently it is difficult to extrapolate to the range of low doses, as required for risk estimates of X-ray diagnosis and industrial radiation exposure. Since estrogenic hormones have been reported to promote radiation-induced carcinogenesis and because a large proportion of women at risk are on contraceptive estrogen medication, it is also important to investigate the combined effects of radiation and estrogens.

Proposed Course: At the time of drafting this contract narrative, 800 rats still under observation. The scoring of tumor prevalence will be continued for hystero-ovariectomized and estrogen-treated animals. All new data are processed on computer in order to enable direct retrieval of the status of the project and to store the final results. At the conclusion of the program, which is foreseen in December 1978, information will be available on latency periods, single or multiple occurrence of tumors, histological type of tumors, nature of dose-effect relationships and RBE for the different neutron beams.

Date Contract Initiated: June 25, 1973

Current Annual Level: $73,000

PASADENA FOUNDATION FOR MEDICAL RESEARCH (N01-CP-65850)

Title: Primary Culture of Normal, Human Prostatic Epithelial Cells.

Contractor's Project Director: Dr. M. Edward Kaighn

Project Officer (NCI): Dr. Stuart H. Yuspa

Objectives: To develop procedures for establishment and characterization of primary cultures of normal, human prostatic epithelial cells.

Major Findings: Several cell lines have been isolated from the normal neonatal prostate. They have the ultrastructural features of epithelial cells and the normal human male karyotype (2N=46), with a fluorescent Y-chromosome. One line (NP-2) has undergone 45 population doublings. It will not grow in soft agar but clones with a 15-20% plating efficiency. 2) A non-enzymatic method was developed to successfully subculture prostate epithelial cells for the first time. The method has been found to be reproducible. Preliminary evidence by scanning electron microscopy indicates significant changes in surface architecture as a result of potassium treatment. 3) Nutritional studies show that nutrient medium, PFMR-1, supplemented with selected fetal calf serum is adequate for isolation and propagation of normal prostate cells. 4) Addition of epidermal growth factor (EGF) and fibroblast growth factor (FGF) to the medium increase both the rate of growth and the lifespan of normal prostate epithelial cells. Titration experiments by clonal methodology in which both the plating efficiency and colony size have been scored show that EGF is effective in the 3-19 ng/ml range whereas experiments still in progress indicate that FGF is optimally effective at higher ranges, of the order of 25 ng/ml or greater. With the NP-2 cell line, these factors act synergistically. This interaction is not seen in prostate-derived fibroblasts. Insulin has no effect on either the clonal growth rate of plating efficiency of this cell line, neither has vitamin A. 5) In the clonal assay, a critical dose-dependence continues to be found for each lot of serum for growth of these cells. Both plating efficiency and colony size are proportional to serum concentration. With optimal lots the serum can

be reduced from 7% to 2.5%. 6) Testosterone (10^{-6} M) stimulates a 4-fold incorporation of tritiated thymidine into the DNA of NP-2 cells but not foreskin fibroblasts of the same neonate. 7) The normal prostate cells have testosterone 5-alpha reductase activity. One of the two NP-2 lines specifically binds labelled testosterone metabolites in the nucleus.

Proposed Course: 1) The culture medium will be improved further to decrease reliance upon serum. 2) The effect of the potassium-passing method at the ultrastructural level of the cells will be studied. 3) Additional epithelial cell lines will be isolated and frozen in liquid nitrogen for future use. 4) Finally, a search for additional criteria for characterization of the cells as originating from the prostate will be continued.

Date Contract Initiated: September 30, 1976

Current Annual Level: $109,600

RESEARCH TRIANGLE INSTITUTE (NO1-CP-75932)

Title: Synthesis of New Retinoids for In Vitro Studies of Prevention of Lung Cancer and Other Epithelial Cancers

Contractor's Project Director: Dr. Frank Ivy Carroll

Project Officers (NCI): Dr. Carl E. Smith
Dr. Michael B. Sporn

Objectives: The objective of this project is to synthesize retinoids possessing 1) effectiveness in controlling cellular differentiation of epithelial tissues, 2) low toxicity, and 3) low tendency for accumulation. To this end and based on published information on the subject, a program has been developed which proposes the synthesis and testing of the following classes of retinoids. 1) Derivatives of 13-cis retinoids; 2) Cyclic derivatives of all trans retinoids; 3) All trans retinoids substituted in the polyene side chain; and 4) Analogs of retinyl amine.

Major Findings: Two heretofore unreported retinoids, all trans-allylretinylether and all trans-15-ethylretinone, have been prepared, characterized and submitted to NCI to be tested for activity in reversal of keratinization of tracheal organ cultures.

Three previously reported compounds have also been prepared, characterized and submitted. They are: 1) All trans-N-n-butylretinylimine, 2) All trans-N-n-butylretinylamine, and 3) 13-cis-12-carboxyretinoic acid anhydride.

In addition, small samples of the following previously unreported retinoids have been prepared and characterized: 1) All trans-15-phenylretinone, 2) 13-cis-15-phenylretinone, 3) All trans-15,15-diphenylretinol, 4)

13-Cis-15-ethylretinone, 5) All trans-methylretinylimine, and 6) All trans-methylretinylamine.

Significance to Biomedical Research and the Program of the Institute: Test results on the compounds submitted are not available at this time.

Proposed Course: Continue to synthesize and submit to NCI for testing the retinoids listed in the Objectives.

Date Contract Initiated: September 30, 1977

Current Annual Level: $116,457

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (NO1-CP-75951)

Title: Studies of Metabolic Capacity in Intestinal Mucosa

Contractor's Project Director: Dr. Lucy M. Anderson

Project Officer (NCI): Dr. Carl E. Smith

Objectives: High risk of cancer of the colon is associated with the high fat-low fiber diet characteristic of industrialized countries. It is important to ascertain whether colon tumors are initiated by chemical carcinogens present in food or arising in the gut contents. In order for chemically stable carcinogens to initiate a neoplasm in the colonic mucosa, they must be enzymatically activated, either by the mucosal cells themselves or by enteric bacteria. The objective of this project is to study the metabolism of several carcinogens by cell-free preparations of rodent and human colonic mucosa. The chemicals to be studied include three environmental carcinogens (benzpyrene, dimethylnitrosamine, and N^6-(methylnitroso)adenosine) and two known colon carcinogens (N-methyl-N'-nitro-N-nitrosoguanidine and dimethylhydrazine). The influence of diet, enzyme inducers, and genetics on metabolism of these compounds will be studied.

Major Findings: The project is at present in the initial stage of development of methodology and establishment of baseline data. Composition of working solutions, procedures for tissue scraping and homogenation, induction schedules, etc., have been systematically tested. Benzpyrene hydroxylase has been found to be induced in colon mucosa of both rats and mice by per os administration of Araclor 1254, a mixture of polychlorinated biphenyls and an environmental contaminant. Appropriate control studies have confirmed the enzymatic nature of the reaction detected. Probable dimethylnitrosamine demethylase activity has been detected at low levels in uninduced colonic mucosa from certain mice.

Significance to Biomedical Research and the Program of the Institute: Knowledge of the ability of colonic mucosa cells to metabolize carcinogens, resulting in their activation and/or detoxification, is essential

to an understanding of the role of chemical carcinogens in the etiology of human colonic cancer.

Proposed Course: During the next six months the baseline studies on benzpyrene hydroxylase in colonic mucosa of rats and mice will be completed and use of this assay extended to human biopsy material, both fresh and after organ culture or nude mouse implant. Studies in which genetics, diet, and/or enzyme inducers are specific variables will be initiated with both rodent and human material. The activity of DMN demethylase will be studied further, and methodology for experiments with the other carcinogens explored.

Date Contract Initiated: September 30, 1977

Current Annual Level: $92,051

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (N01-CP-75950)

Title: Studies of Metabolic Capacity in Intestinal Mucosa

Contractor's Project Director: Dr. M. Earl Balis

Project Officer (NCI): Dr. Carl E. Smith

Objective: This is a program designed to ascertain the interactions among the many factors that contribute to colon cancer. A considerable body of data has been acquired that indicates a role of diet, reducing agents and endogenous or exogenous chemicals on the incidence of the disease. It is the goal of this program to identify the significance of each factor and their interdependence. The methodology is to see if changes brought about by known carcinogens can be used as criteria of carcinogenic potency of other stresses. It is further hoped that assay of relative changes in biochemical parameters in the dividing and differentiated cells will provide a useful screen of potential colon carcinogens. Emphasis will be placed on early changes. Success in this would permit a simple means of detecting potential carcinogens and effectors of other carcinogens.

Major Findings: The initial work was aimed at establishing baseline parameters of cell division in control rats.

1) 5-phosphoribosyl-1 pyrophosphate synthetase (PRPP synthetase) has been reported as a cytosol enzyme in human erythrocytes. On the other hand, studies in this laboratory on the subcellular enzyme distribution of PRPP synthetase in rat tissue cells have revealed that PRPP synthetase activity is totally membrane associated.

2) Three of the naturally occuring polyamines, i.e., spermine, spermidine and putrescine have been found to interact with PRPP by forming a tightly bound complex with the phosphate compound. An interferance by the poly-

amines on PRPP utilizing enzyme activities was expected and later confirmed in this laboratory.

Significance to Biochemical Research and the Program of the Institute:
1) One of the early events that occurs upon stimulation of cell division is the alteration of cellular level of cyclic nucleotides. Recently, observations of the following have been reported: a) a rapid increase of PRPP concentrations after PHA stimulation of lymphocytes; and b) PRPP synthetase activity was allosterically regulated by cyclic purine nucleotides. These observations strongly suggest that the regulation of PRPP systems may be critically related to the control of cyclic nucleotides production, and an important regulatory role of these enzymes in cell growth and differentiation is expected. The finding from this laboratory that the PRPP synthetase activity of tissue cells are tightly associated with membrane further emphasizes the significance of the role of PRPP metabolism.

2) PRPP is a compound that is at a focal point of several metabolic reaction sequences. It is known that strong interactions exist between Mg^{++} and PRPP. The PRPP-Mg complex is the true substrate for enzymes that utilize this compound. The finding from this laboratory that the naturally occuring polyamines, i.e., spermine, spermidine and putrescine interact with PRPP in the way that Mg^{++} does indicates that there is a regulatory function of polyamines upon the PRPP utilizing reaction. This is of particular importance since the total intracellular concentrations of these polyamines reach millimolar levels.

Proposed Course: To continue the earlier proposed studies and to compare the subcellular PRPP synthetase activity distribution profile of the carcinogen-induced tumor cell to that of its origin. Similar approach will also be applied to other enzymes. Studies will be emphasized on the elucidation of the role of polyamines on cell differentiation and transformation, and to interrelate the regulatory functions of polyamines to other metabolic pathways.

Date Contract Initiated: September 30, 1977

Current Annual Level: $90,170

SOUTHERN RESEARCH INSTITUTE (NO1-CP-75914)

Title: Isolation, Identification, and Culture of Epithelial Cell Types from the Colon of Experimental Animals

Contractor's Project Director: Dr. D. P. Chopra

Project Officer (NCI): Dr. Gary Stoner

Objectives: The establishment of homogenous populations of stem and differentiated cells from normal colonic epithelium will facilitate

studies on the carcinogenesis of the colon. This project has three main objectives: (1) to obtain profilerating and differentiated cell populations; (2) to identify these cells as normal, epithelial in nature and colonic in origin; and (3) to show that the isolated cells are viable and can be maintained in culture. However, before attempting separation and identification of stem and differentiated cells it is necessary to obtain single cell suspensions.

Major Findings: The stem and differentiated cells of the colonic epithelium are arranged in relatively demarcated zones. A method has been developed consisting of repeated, timed enzymatic dissociations to gradually dissociate the colonic epithelium to obtain the two cell types.

Four single cell suspensions with a total of approximately $2.5x10^7$ cells were obtained from the colon of a single Buffalo rat. Scanning electron microscopic observations of the four suspensions showed that suspensions one and two obtained after the first two enzymatic dissociations contained cells with extensively folded surfaces while suspensions three and four obtained after the later two enzymatic dissociations contained cells that were round and had smoother surfaces. When the rat was pulse-labeled with tritiated thymidine (H^3Tdr) prior to cell dissociation, a significantly greater amount of H^3TdR was incorporated into cells present in suspensions three and four than in suspensions one and two. The data suggest that suspensions one and two that were obtained from the luminal half of the epithelium essentially contained differentiated cells while suspensions three and four obtained from deeper zones of the epithelium contained essentially proliferating cells.

Significance to Biomedical Research and the Program of the Institute: The primary goal of the carcinogenesis program of the National Cancer Institute is to prevent cancer in man by identifying environmental carcinogenic factors that may affect humans, as well as by identifying the mechanisms of action of known carcinogens. Several cell and organ culture models for epithelial cancers have been developed and these models have led to important findings in several aspects of carcinogenesis. Recently NCI has been emphasizing the use of human tissues for studies of carcinogenesis in organ systems at high risk for cancer in man. For this purpose NCI is interested in developing methods to isolate specific cell populations from surgical samples of human colon. Since the amounts of human tissues available for experimental use are limited, contractor's efforts are focused to develop the necessary methodology for isolation, identification, and culture of specific cell types from the normal colon of Buffalo rat. The methods developed in the rat model will be applied to isolate specific cell types from surgical samples of human colon.

Proposed Course: The cell dissociation procedure outlined above will continue to be used to obtain single cell suspensions from rat colon. Preparations enriched in stem cells and differentiated cells will be obtained by use of the Ficoll gradient procedure. The different cell

types will be identified and characterized by scanning and transmission electron microscopy, biochemical and immunological methods. After identifying and characterizing stem and differentiated cells, tissue culture media and conditions suitable for maintaining these cell types in vitro will be developed.

Date of Contract Initiated: September 29, 1977

Current Annual Level: $96,480

SOUTHERN RESEARCH INSTITUTE (NO1-CP-22064)

Title: Organ Culture Assay of Vitamin-A Analogs

Contractor's Project Directors: Dr. Lee J. Wilkoff
Dr. Donald L. Hill

Project Officer(NCI): Dr. Michael B. Sporn

Objectives: The objectives of this contract are (1) to develop adequate bioassays to evaluate the biological activity of new retinoids as measured by control of epithelial differentiation; (2) to perform bioassays on selected retinoids; (3) to develop new quantitative assays for retinoid activity in organ or tissue culture; (4) to evaluate the morphological, histological, and biochemical aspects of toxicity and pharmacology of selected retinoids to rodents; (5) to investigate the biochemical changes caused by carcinogens in target tissues and their possible reversal by retinoids; and (6) to synthesize a limited number of retinoid compounds (as directed by the Project Officer) which have relatively well-defined synthetic methods.

Major Findings: Selected retinoids were evaluated for their effect in reversing N-methyl-N-nitro-N-nitrosoguanidine-induced hyperplasia in mouse prostate organ cultures. The 14-fluoro analog of ethyl retinoate was as active as β-retinoic acid. Other retinoids tested were less active than β-retinoic acid. 13-cis-Retinoic acid, the TMMP analogs of retinoic acid, N-ethylretinamide, and the 1-methoxy-ethylcyclopentenyl analog of retinoic acid were about as active as β-retinoic acid in reversing testosterone-induced hyperplasia in mouse prostate organ cultures. The 1-methoxyethylcyclopentenyl analog of retinoic acid was approximately tenfold more effective than β-retinoic acid in modulating epithelial differentiation of 13-day chick embryo metatarsal skin explants, whereas the dichlorophenyl analog exhibited about the same activity as β-retinoic acid. Other retinoids tested were less active than β-retinoic acid in altering epithelial differentiation of 13-day chick skin explants. Exposure of hyperplastic and preneoplastic bladder explants from rats previously treated with N-butyl-N-(4-hydroxybutyl) nitrosamine to 1.7×10^{-5}M 13-cis-retinoic acid had apparent reparative effect on epithelial morphology.

A retinoic acid-binding protein (RABP), which may mediate the function of retinoic acid by serving as its cellular receptor, is present in various epithelial tissues of animals. RABP has been found in the nuclei as well as the cytoplasm of cells of chick embryo skin and may be a membrane-bound receptor. Although the binding affinity of RABP for β-retinoic acid and its synthetic analogs correlates well with their biological activity, no such correlation can be established for their binding to serum albumin, the protein involved in transport of retinoic acid and its analogs in blood. RABP has been purified to homogenity, as judged by its electrophoretic mobility on polyacrylamide gels; and involvement of thiol groups in the binding of retinoic acid has been demonstrated. RABP is present in several transplantable tumors and in lungs to which RABP-containing tumor cells have metastasized.

Subchronic toxicity studies in rats showed that doses of 13-*cis*-retinoic acid three to five times higher than those of all-*trans*-retinoic acid were required to produce increases in concentrations of plasma alkaline phosphatase and decreases in concentrations of hemoglobin. Dose-related decreases in concentrations of plasma albumin were observed in rats treated with either retinoid. The results indicate that 13-*cis*-retinoic acid may be more useful clinically than all-*trans*-retinoic acid. Subacute toxicity studies of *N*-ethylretinamide, *N*-hydroxyethylretinamide, and *N*-hydroxyphenyl-retinamide in mice show that these compounds are not very toxic. Preliminary results indicate that the nonsteroidal anti-inflammatory agents, Ibuprofen and Indomethacin, are more effective than aspirin in protecting mice against bone fractures produced by sublethal retinoid intoxication.

The amounts of 13-*cis*-retinoic acid that appear in serum of mice, rats, and hamsters, after oral or i.p. administration, are greater than those of all-*trans*-retinoic acid, suggesting that the toxicity of retinoids is due to factors other than absorption into the blood stream.

Treatment of rats with all-*trans*-retinoic acid resulted in an increase of serum *N*-acetyl-β-*D*-hexosaminidase activity.

N-Ethylretinamide becomes tightly, but reversibly, bound to rat liver microsomes. This binding is inhibited strongly by retinal and retinoic acid but not by retinyl acetate or methyl retinoate. These results are consistent with microsomal metabolism of *N*-ethyl-retinamide.

High pressure liquid chromatography procedures have been developed and used to detect submicrogram amounts of all-*trans*-retinoic acid, 13-*cis*-retinoic acid, and the ethyl amide and hydroxylethylamide of retinoic acid in the serum of rats.

N-Ethylretinamide, *N*-(2-hydroxyethyl)retinamide, *N*-methyl-retinamide, *N*-retinoylglycine, and *N*-retinoylglycine ethyl ester have been synthesized in quantities in excess of one kilogram for extensive biological evaluation.

Significance to Biomedical Research and the Program of the Institute: Vitamin-A is required for normal differentiation and growth of epithelium in the following organs, all of which are major sites of primary cancer in man: bronchi and trachea, colon, stomach, uterus, kidney and bladder, testis, and skin. Vitamin-A and other retinoid compounds are also able to alter the process of chemical carcinogenesis in several different epithelia and to block the effects of several different carcinogens. Retinoids can inhibit carcinogenesis even when administered several weeks after the carcinogen. Studies are currently in progress in the Carcinogenesis Program to elucidate the role of retinoids in this process. This effect of retinoids is presumed to be related to the biochemical and cellular role of retinoids in determining normal differentiation in these tissues.

The ultimate implications of these studies on the inhibition of epithelial carcinogenesis are important. If vitamin-A is indeed functioning as a differentiation hormone, it should be possible, with the cooperation of organic chemists, to design and synthesize new retinoids having even greater potency than vitamin-A on differentiation of epithelia. The precedents in steroid chemistry for this approach to vitamin-A are impressive; presently there are many synthetic steroid analogs that are more potent (and often more specific and less toxic) than the naturally occurring estrogens, progestational agents, androgens, or adrenocortical steroids. If this type of approach can be applied to the hormonal action of vitamin-A, the potential for inhibiting carcinogenesis in many types of epithelia would be greatly increased.

Proposed Course: 1) Continue the development of bioassay methods using organ culture systems to evaluate the biological activity of retinoids. An additional organ culture system involving tracheobronchial epithelial tissue derived from vitamin-A deficient hamsters has been developed and will be used for the primary evaluation of newly synthesized retinoids. 2) Devise new methods, using morphological, histochemical, and biochemical end points for quantitative bioassay of retinoids. For instance, high pressure liquid chromatographic analysis for tissue and blood levels of retinoids will be used. 3) Determine the biochemical characteristics and biological role of retinoic acid-binding protein, and attempt the development of a biochemical screen for retinoid activity using this protein. 4) Continue the evaluation of the morphological, histological, and biochemical aspects of toxicity and pharmacology of selected retinoids in rodents. 5) Study the biochemical changes caused by carcinogens in target tissues and their possible reversal by retinoids. 6) Synthesize a limited number of retinoids as directed by the Project Officer.

Date Contract Initiated: December 10, 1971

Current Annual Level: $516,454

SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION (N01-CP-33379)

Title: Studies of Hormonal Factors of the Human and Animal Prostate

Contractor's Project Director: Dr. Sydney A. Shain

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The relationship between aging-associated changes in endocrine regulation of prostate function and the pathogenesis of prostate adenocarcinoma is ill-defined and poorly understood. The principal objectives of this program have been the interage and interspecific evaluation of prostate androgen metabolism and the cellular content, distribution, and specificity of prostate androgen receptors in normal, lesion-free senile and neoplastic specimens of rodent, canine, and human prostate. These components of prostate cellular metabolism were chosen as they are either known or thought to be intimately involved in the regulation of prostate eipthelial cell function. During the course of these studies spontaneous adenocarcinomas of the AXC rat ventral prostate were detected with a frequency of 70% in rats greater than 30 months of age. Moreover, specific aging-associated changes were identified in canine and rat prostate androgen metabolism and androgen receptor content and distribution. These observations provided biochemical indices of prostatic senility and supported a proposal of a mechanism for the induction of precocious prostatic senility as a model for studying factors promoting prostate carcinogenesis.

Major Findings: Completed evaluations of testosterone metabolism by the prostate of the aging canine demonstrated that the aging-associated shift to increasingly reductive testosterone metabolism was composed of two components: 1) an aging-related increased production of 5α-dihydrotestosterone and 2) an aging-related decreased production of 4-androstenedione. This shift in testosterone metabolic capacity is opposite to that characteristic of the aging rat prostate which develops spontaneous adenocarcinoma.

Continuing evaluations of testosterone metabolism by early passage transplantable rat prostate adenocarcinomas demonstrate enhanced oxidative testosterone metabolism relative to normal senescent rat ventral prostate and comparable oxidative testosterone metabolism relative to primary rat ventral prostate adenocarcinomas. The data suggest oxidative testosterone metabolism to be characteristic of preneoplastic and early adenocarcinomatous rat ventral prostate.

Continuing evaluations of testosterone metabolism by human benign hyperplastic prostate (BPH) demonstrates the reductive testosterone metabolic capacity of this tissue to be greater than that of either senescent canine or rodent prostate. Human BPH was the only tissue in which in vitro exposure to estradiol caused a shift to oxidative testosterone metabolism which was principally attributable to decreased elaboration of 5α-dihydrotestosterone.

Completed evaluations of prostatic androgen receptor content in tissue from 2.5-, 4.5-, and 11.1-year-old purebred dogs demonstrated the absence of an aging-associated decrease in receptor content per prostate cell. The result

was in complete opposition to that which characterizes the prostate of the aging rat.

Procedures were developed for the reliable determination of occupied (RA) and unoccupied (R) cytoplasmic and nuclear androgen receptors in human prostate specimens. The protocols provide reliable analyses with as little as 300 mg of tissue.

Medrogestone was demonstrated to be a highly effective antigonadal agent for the production of precocious prostatic senility. Chronic treatment caused prostatic involution and decreased androgen receptor content in prostate of 5-month-old rats to levels which were comparable to those of 18- to 20-month-old rats. The changes were not due to preferential loss of epithelial cells.

Melatonin was demonstrated to be a weakly antigonadal agent in light, 15:9/L.D., adapted young mature AXC rats. The effect of melatonin was indicated to be due to altered differentiated cell function.

Significance to Biomedical Research and the Program of the Institute: The elucidation of the alterations in prostate homeostasis which may be related to the pathogenesis of prostatic adenocarcinoma will facilitate the determination of possible causes of and provide for the development of methodology for early detection of risk of prostate adenocarcinoma. Identification of causative factors will allow for rational endocrine manipulation in an attempt to reduce the incidence, mortality, and morbidity of prostate cancer.

Proposed Course: This project will continue comparative evaluations of aging-related changes in specific components associated with hormonal regulation of prostate epithelial cell function. These studies will include continuing evaluations of androgen metabolism in normal, hyperplastic, and adenocarcinomatous human prostate and examination of hormone receptors in these tissues. The model for precocious prostatic senility will be evaluated for the early induction of adenocarcinoma of the rat ventral prostate.

Date Contract Initiated: June 1, 1973

Current Annual Level: $350,000

STANFORD RESEARCH INSTITUTE (N01-CP-75936)

Title: Synthesis of Radioactive Retinoids for Metabolic and Pharmacologic Studies Relating to Prevention of Lung Cancer and Other Epithelial Cancers

Contractor's Project Director: Dr. Joseph I. DeGraw

Project Officers (NCI): Dr. Charles Frolik
Dr. Anita Roberts

Objectives: The objective of the research is to synthesize adequate quantities of radioisotopically labeled retinoid compounds. These are to be used as tracers in the investigation of the metabolic and pharmacologic action of retinoid compounds as anticarcinogenesis agents.

Major Findings: The synthesis of 5mCi of ^{14}C-labeled retinylidineacetylacetone was completed. The penultimate intermediate for the preparation of 11,12-ditritioretinol, a required intermediate for 11,12-ditritioretinylacetate and 11,12-ditritioretinylidineacetylacetone, has been prepared via a four-step synthesis from β-ionone. The conversion of unlabeled retinol to retinylidineacetylacetone has been successfully accomplished.

Significance to Biomedical Research and the Program of the Institute: Retinoid deficiency enhances the susceptibility of experimental animals to chemical carcinogenesis. The application of retinoids can reverse hyperplasia or lesions induced by carcinogens. The mechanism of action by which retinoids manifest this effect is largely unknown. The use of radiolabeled retinoid compounds is required for a study of their metabolism and mechanism of action. Such studies may enable investigators to design more prophylactically useful retinoids suitable for clinical application in cancer prevention.

Proposed Course: The preparation of the 11,12-ditritio compounds discussed above will be completed via introduction of tritium into the key intermediate. In addition, another labeled material, retinylidinecyclohexandione, is under preparation.

Date Contract Initiated: September 30, 1978

Current Annual Level: $86,726

STANFORD RESEARCH INSTITUTE (NO1-CP-75931)

Title: Synthesis of New Retinoids for In Vitro Studies of Prevention of Lung Cancer and Other Epithelial Cancers

Contractor's Project Director: Dr. Marcia I. Dawson

Project Officers (NCI): Dr. Carl E. Smith
Dr. Michael B. Sporn

Objectives: Contractor is synthesizing retinoids that may have better pharmacological properties than those that have already been tested. These analogs have both steric and electronic modifications in the ring, side chain, and polar terminus of the retinoid skeleton.

Major Findings: Thus far, contractor has prepared the following retinoids: 2-(2'-methoxyethoxy)ethyl retinoate; N-2-(2'-methoxyethoxy)ethyl retinamide;

ethyl all-trans-2,7-dimethyl-9-(2'-norbornenyl)-2,4,6,8-nonatetraenoate; all trans-1-(3'-hydroxyphenyl)-4-methyl-6-(2',6',6'-trimethyl-1'-cyclohexen-1'yl)-1,3,5-hexatriene; all-trans-1-(3'-acetoxyphenyl)-4-methyl-6-(2',6',6'-tri-methyl-1'-cyclohexen-1'-yl)-1,3,5-hexatriene; all-trans-1-(3'-methoxyphenyl)-4-methyl-6-(2',6',6'-trimethyl-1'-cyclohexen-1'-yl)-1,3,5-hexatriene; all-trans-1-(3'-acetamidophenyl)-4-methyl-6-(2',6',6'-trimethyl-1'-cyclohexen-1'-yl)-1,3,5-hexatriene; and all-trans-1-(3'-ethylaminophenyl)-4-methyl-6-(2',6',6'-trimethyl-1'-cyclohexen-1'-yl)-1,3,5-hexatriene. These compounds will be submitted for testing shortly.

Significance to Biomedical Research and the Program of the Institute: Retinoid deficiency enhances the susceptibility of the epithelial tissue of the colon, bladder, and lung of experimental animals to chemical carcinogenesis. Synthetic retinoids can inhibit the development of epithelial cancer of the skin, respiratory tract, mammary gland, and bladder in experimental animals and can reverse the hyperplasia induced by chemical carcinogens in prostatic and tracheal organ cultures. Drug development in this area is required because of the limited usefulness of the natural retinoids due to their inherent toxicity and tissue distribution limitations. Synthetic efforts must be aimed at the development of nontoxic drugs that could be used on a chronic basis for the augmentation of normally operative body mechanisms that arrest or reverse preneoplastic processes during the progression to invasive malignancy.

Proposed Course: 1) To continue the preparation of retinoid analogs, making use of any structure-activity data that become available for the design of future compounds; and 2) to synthesize more polar retinoids that may localize in the bladder and therefore be effective against bladder cancer.

Date Contract Initiated: September 30, 1977

Current Annual Level: $139,161

STATE UNIVERSITY OF NEW YORK (At Stony Brook) (N01-CP-33361)

Title: Studies of Carcinogenesis in Organ Culture of Trachea and Bronchi

Contractor's Project Director: Dr. Bernard P. Lane

Project Officer (NCI): Dr. Michael B. Sporn

Objectives: 1) To determine the optimal conditions for the organ culture of tracheal rings; 2) to observe the biology of tracheal ring cultures; 3) to determine the effects of exposing tracheal rings to the carcinogen benzo[a] pyrene (BP) in vitro; and 4) to monitor cultures exposed in vitro for malignant transformation.

Major Findings: 1) Methods for large-scale long-term culture of rat tracheal tissues were developed during past contract periods. McCoy's 5a

(modified) medium buffered to pH 6.8-7.3 with sodium bicarbonate and maintained in an atmosphere of 5% CO_2 with air will support growth of tracheal epithelium, and studies of epithelial growth and maintenance of differentiation in CMRL 1066, Parker's 199 and Dulbecco's modification of Eagle's MEM demonstrated that these alternative culture media offer no advantage over McCoy's 5a (modified) medium. Using a mechanical slicing device, cultures are prepared in batches of 300-500 and grown under these conditions for periods of months. The cultures are uniform in appearance and in the ability to respond to various stimuli by cellular repair and by mitotic activity.

2) A study of the role of serum in epithelization of the free floating culture, using light, transmission electron and scanning electron microscopy, established the basis for observed differential patterns of the appearance of ciliated cells and dividing cells on the surfaces of the culture. Serum was shown to facilitate migration of the epithelial cells as a continuous flattened sheet. The cells do not divide while migrating. These results parallel events in tracheal epithelial wound healing in vivo. After epithelization of the surface of the ring is complete, wounding in vitro results in focal response similar to that seen in vivo. In the absence of wounding, the proliferative rate of cultured epithelium is 3-4%, many times that of normal epithelium in situ, but the populations participating in the cell renewal are the same as those engaged in slow turnover in vivo. Addition of retinyl acetate at concentrations up to 0.5 μg/ml of medium increases degree of cellular differentiation but concentrations above this level are toxic.

3) A series of protocols for exposure of the cultures to carcinogens were carried out. Continuous exposure to BP in the culture medium was found to result in shortening of the life of the culture in rough proportion to the concentration of this carcinogen. A maximal effect, consisting of hyperplasia, after two weeks of exposure to 2 μgm/ml BP gives way to atrophy if treatment is continued for several weeks longer. To avoid the apparent masking of carcinogenicity by cytotoxicity of the agent, two modified exposure regimes were evaluated. When 30-hour exposure periods were alternated with 72- to 138-hour periods in medium lacking the carcinogen, cell death occurred as observed with continuous exposure. However, cultures exposed continuously for two weeks to 2μgm/ml and then removed to carcinogen-free medium developed areas of focal hyperplasia, which may be equivalent to nodular hyperplasias in livers of animals treated with carcinogens. They persist for 6 or more weeks and are reversed or prevented from appearing by addition to the medium of their progeny as well as selection of specific populations.

The ultrastructure of carcinogen-treated cultures has been studied to identify markers of malignant transformation before diagnostic histologic changes appear. Lanthanum staining reveals extensive tight junctions in the epithelium and there are structural abnormalities of the plasma membranes whose characteristics are being studied. The appearance of

abnormal structural proteins and enzymes and deviations from normal cell cycle kinetics are also being assessed. Comparisons are made with untreated cultures, normal tracheal epithelium, regenerating tracheal epithelium, tracheas treated in vivo with benzo[a]pyrene, and tumors arising in tracheas in vivo.

Cells are grown in monolayer cultures derived from carcinogen-treated cultures. The growth characteristics, ultrastructure and cytogenetics of these cells are compared with those from normal freshly excised tissues and those from untreated cultures. After carcinogen exposure, explantation is more vigorous and the cells are pleiomorphic. Tumor formation by this population will be studied when a stable expandable line is established.

4) Previous work established that Fischer rats were suitable as both donor and host for studies involving implantation of carcinogen-treated organ cultures into intact animals to test their biologic potential. Cultures exhibiting focal epithelial hyperplasia at the time of implantation continued to exhibit this feature on removal two weeks later. Those retrieved later than two weeks after implantation had reverted to normal histology. The ability of croton oil to promote malignant progression of epithelium in implanted carcinogen-treated cultures is being tested by injection of this known promoter of skin tumors near the site of implanted cultures. No tumors have appeared but there is increase in the length of the period of normal histology. Experiments in which croton oil is applied in a controlled and sustained manner are now under way.

Cultures have also been implanted in neonatally thymectomized syngeneic rats in order to test whether cell mediated immunity plays a role in the disappearance of the carcinogen-induced histologic changes. Since the implanted cultures revert to normal in the same period as they do with intact hosts, a set of experiments is now under way in which the cultures are implanted in the anterior eye chamber of rabbits, an immunologically sequestered site.

In addition to the above experiments directed toward recognition of required cofactors and/or host responses, carcinogen-treated cultures are being implanted in large numbers into syngeneic intact animals to look for low-incidence tumorigenic behavior.

Significance to Biomedical Research and the Program of the Institute:
The tracheobronchial epithelium gives rise to more fatal human tumors than any other tissue. Yet experimental access to many parameters of carcinogenesis under controlled conditions, including dependable tests of malignant transformation, has been limited. Long-term organ culture of tracheobronchial epithelium constitutes a model which preserves the biologic organization of the tissue while permitting control of almost all variables affecting population kinetics and cellular differentiation, stages in carcinogenesis and modification of carcinogen effects by other agents. This contract is directly concerned with the problems of determination of causes of lung cancer, with the early diagnosis of lung cancer lesions, and with effective prevention of lung cancer.

Proposed Course: Carcinogen exposed cultures will be implanted in large numbers on syngeneic and immunocompromised hosts with and without in vitro and in vivo treatment with croton oil and its tumor promoting components. Cells from cultures which have been incubated in medium containing BP will be grown in monolayer cell culture and implanted in large numbers as a further test for tumorigenicity.

In addition to tests for biologically malignant behavior, the cell populations produced by in vivo exposure to BP will be compared structurally and functionally to the various cell types in normal, regenerating and and Vitamin-A deficient epithelium and to cells of tumors induced in this tissue by in vivo exposure to the same carcinogen. These studies will help elucidate the place in the process of carcinogenesis of the observed reproducible changes in epithelium exposed to BP in vitro.

Date Contract Initiated: June 25, 1973

Current Annual Level: $129,385

STOCKHOLM, UNIVERSITY OF (NO1-CP-33363)

Title: Studies of Polycyclic Hydrocarbon Metabolism in the Respiratory Tract

Contractor's Project Director: Dr. Lars Ernster

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The objective of this contract is to define the relationship between metabolism of polycyclic hydrocarbons and pulmonary neoplasia. Specific aims are: 1) the determination of the cellular distribution of aryl hydrocarbon monooxygenase and of its substrates and products in pulmonary tissue; 2) subcellular fractionation of lung cells and subcellular localization of aryl hydrocarbon monooxygenase activity; 3) development and employment of a highly sensitive assay for aryl hydrocarbon monooxygenase, including studies of tissue disruption to optimize enzymatic activity accessible to assay; 4) dissociation of the cells of the lung and isolation of individual cell types; 5) characterization of the lung aryl hydrocarbon monooxygenase system, including induction of this system, identification of the metabolites of polycyclic hydrocarbons in pulmonary tissue, and characterization of the finding of these metabolites to DNA; 6) isolation and characterization of lung cytochrome P-450; 7) improvement of the assay for pulmonary epoxide hydrase activity and characterization of this enzyme in much the same manner as aryl hydrocarbon monooxygenase was characterized; 8) improvement of the assay for pulmonary glutathione S-epoxide transferase and isolation and characterization of this enzyme; 9) studies on polycyclic hydrocarbon metabolism in isolated intact hepatocytes and lung cells; 10) studies on the manner in which reactive metabolites of polycyclic hydrocarbons travel from the site of generation to the "target site"; and 11) initial studies on the metabolism of polycyclic hydrocarbons in human lung and skin.

Major Findings: 1) A sensitive and convenient radioactive assay for benzpyrene monooxygenase has been developed based on the use of tritium-labeled substrate and separation of substrate from products by a simple extraction procedure. 2) The applicability of a direct fluorometric assay for benzpyrene monooxygenase to rat lung tissue has been investigated. 3) The new radioactive assay has been employed in studies of various aspects - including kinetics, inhibition, effect of inducers, nature of the products - of benzpyrene metabolism by isolated intact rat hepatocytes. 4) The fluorometric assay has been used to investigate the kinetics and inducibility of benzpyrene monooxygenase in rat lung. 5) A method has been developed for removing hemoglobin from lung microsomes by chromatography on Sepharose, and another method has been developed to allow the measurement of cytochrome P-450 in the presence of hemoglobin. 6) The spectral properties of cytochrome P-450 have been investigated in lung microsomes from which hemoglobin has been removed. 7) Lung cytochrome P-450 has also been isolated and the properties of the purified form studied. 8) Subfractionation studies on lung microsomes have been carried out involving selective aggregation of fragments of the endoplasmic reticulum with cesium ions. 9) A sensitive modified assay for epoxide hydrase has been developed and used to characterize this activity in lung tissue. 10) Published assay procedures for glutathione S-epoxide transferase have been made more sensitive and isolation of this enzyme from lung has been started. 11) Levels of glutathione and of various glutathione-metabolizing enzymes in the lung have been measured and compared to the corresponding levels in liver. 12) Lung microsomes have been shown to metabolize 3-hydroxybenzpyrene to a product that binds covalently to DNA. 13) Liver nuclei have been shown to metabolize benzpyrene to products which bind covalently to DNA and these products have been identified. 14) Isolated hepatocytes have also been shown to metabolize benzpyrene to products which bind covalently to DNA and these products have also been identified. The effect of various inhibitors on this binding has been investigated in order to evaluate the relative importance of different detoxification pathways. 15) Human lung microsomes have been isolated and characterized in terms of their cytochrome P-450 system and metabolism of polycyclic hydrocarbons.

Significance to Biomedical Research and the Program of the Institute: Many of the foreign compounds which enter the human body do so through the lungs. Among these are the polycyclic hydrocarbons, which may be major causes of some human cancers. The principal pathway for metabolism of the polycyclic hydrocarbons in mammalian tissues involves the inducible enzyme system aryl hydrocarbon monooxygenase, epoxide hydrase, and glutathione S-epoxide transferase. This contract and others in the program are directed towards a detailed understanding of the nature and function of these enzymes and their relationship to carcinogenesis in the respiratory tract.

Proposed Course: 1) To isolate different cell types from rat lung in order to ascertain the cellular distribution of the different enzymes of polycyclic hydrocarbon metabolism and to study this metabolism under more

metabolite complexes whose formation is catalyzed by isolated nuclei and hepatocytes, as well as to investigate the repair of these complexes; 3) to further characterize the release of benzpyrene metabolites from isolated hepatocytes; 4) to isolate epoxide hydratase using affinity chromatography and to discover its "natural" substrate; and 5) to use a reconstituted system composed of microsomes and isolated glutathione S-transferases to investigate the relative importance of different pathways for transforming arene oxides into less carcinogenic products.

Date Contract Initiated: June 25, 1973

Current Annual Level: $195,000

TEXAS, UNIVERSITY OF (Southwestern Medical School at Dallas) (NO1-CP-33362)

Title: Polycyclic Hydrocarbon Metabolism in the Respiratory Tract

Contractor's Project Director(s): Dr. Russell A. Prough
Dr. Ronald W. Estabrook

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The aim of this contract is to characterize the monooxygenases in lung from susceptible rodents and humans. Since these monooxygenases are capable of activating carcinogenic compounds to proximal and ultimate carcinogens, the enzymatic capacity of lung to metabolize polycyclic hydrocarbons is of considerable importance. The activities measured will be used to choose those activities in lung which can be used to study populations of whole cells derived from lung.

Major Findings: One major area investigated during the current contract period has been the comparison of the lung monooxygenases of hamsters, humans, and rat. Human tissue was obtained at autopsy 1-4 hours after death. The liver and lung metabolism of benzo(a)pyrene (B[a]P) was measured and noted to be lower than other monooxygenase activities. The rate of B(a)P metabolism by human liver was 15% that of rodent liver while lung B(a)P metabolism in humans was nearly identical with the metabolic activity of rodent lung. The proteins involved in foreign compound metabolism in lung microsomal fractions were measured by enzyme assay; the content of cytochrome P-450, the terminal oxidase, was very low in rodent lung and not detectable in human lung fractions.

Analysis of the B(a)P metabolite ratio showed no statistical differences in benzo-ring product distribution (7,8 dihydrodiol or 9,10-dihydrodiol) between rodent lung and liver or human liver. However, the benzo-ring products produced by human lung microsomal fractions were 18% of total metabolites compared to 11-12% for rodent lung fractions. The metabolism of the 7,8-diol has been shown by several groups to yield an ultimate carcinogenic form of B(a)P, the 7,8 diol-9,10-oxide. An increased proportion of 7,8-diol product, a more complete carcinogen than B(a)P, might

lead to an increased susceptibility to B(a)P carcinogenesis in species capable of producing a larger fraction of proximal carcinogen. Capdevila et al., (Biochem. Biophys. Res. Commun. 65:894,1975), have suggested that lung monooxygenases may effectively metabolize B(a)P-phenol derivatives to chemical species capable of alkylating DNA. Contractor's current studies have detected the metabolic products of phenol metabolism using spectrophotometric and HPLC techniques; the steady state products appear to be diphenols (dihydroquinones). The metabolic profile for the 3- and 9-B(a)P-phenols with exogeneous DNA is being evaluated.

Significance to Biomedical Research and the Program of the Institute: One of the scientific methods used to understand the molecular basis of disease has been to elucidate the biochemical events leading to a disease state. This approach will hopefully allow biomedical research scientists to alter the events which are involved in initiation of the process. The lung has been shown to be uniquely susceptible to polycyclic hydrocarbon carcinogenesis. The current studies will evaluate the reactivity of other B(a)P metabolic products and provide basic information which would allow an assessment of the capacity of other B(a)P products to alter cellular nucleophiles. It should be noted that such an approach was used by others to study the generation of diol-epoxides of B(a)P, a possible ultimate carcinogen.

Proposed Course: The contract work schedule will continue the detailed study on how the lung monooxygenases metabolize B(a)P phenols and how reactive these products may be. The human lung monooxygenase will also be studied.

Date Contract Initiated: June 20, 1973

Current Annual Level: $53,500

TORONTO, UNIVERSITY OF (NO1-CP-75879)

Title: Biochemical and Morphological Components of Hepatic Carcinogenesis

Contractor's Project Director: Dr. Emmanuel Farber

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The major objectives are: 1) to purify and characterize a component in the endoplasmic reticulum of preneoplastic hepatocytes, called Preneoplastic Antigen (PN-antigen) that seems to be special to this population; and 2) to develop a novel, short-term assay for chemical carcinogens generally using the appearance and number of putative initiated liver parenchymal cells as an end point.

Major Findings: PN-antigen has been purified from hyperplastic liver nodules. This endoplasmic reticulum component is a glycoprotein, molecular

weight 140,000 daltons, composed of two identical subunits each with serine as the amino terminal amino acid. It is present in a different form in normal liver but remains undetectable until ribosomes are removed from the membranes.

Contractor is progressing well with a new approach to the analysis of the sequence of steps in chemical carcinogenesis in vivo. The first biological step appears to be the induction by a carcinogen of random rare hepatocytes that are resistant to the inhibitory effect of carcinogens on cell proliferation. These putative initiated hepatocytes can now be assayed in vivo and in vitro. The initiation process is a two-step one in which a round of cell proliferation is required as the second step, following the induction of one or more molecular events. In the in vivo assay, the initiated cells can be induced to proliferate very rapidly without proliferation of the surrounding liver cells and thus can be visualized within a matter of days. The use of the relatively specific marker for these cells, gamma-glutamyl transpeptidase, facilitates quantitation both of the number of foci of resistant liver cells and the number of such cells in the whole liver.

Carcinogens not carcinogenic for the adult rat liver, such as 3-methylcholanthrene,7,12-dimethylbenzanthracene, N-methyl-N'-nitro-nitrosoguanidine (MNNG) and N-methylnitrosourea, as well as many liver carcinogens, are all positive with this new assay. Several noncarcinogenic hepatotoxins are negative.

It should be pointed out that liver preparations are required in many different assays, either in prokaryotes or in eukaryotes, for activation of carcinogens. The use of intact liver as both activator and indicator or end-point is rational and attractive. This, coupled with the use of an end-point that has known relevance to cancer, makes this novel approach attractive.

Significance to Biomedical Research and the Program of the Institute: There is general agreement that a need exists for a rapid or short-term assay in vivo for chemical carcinogens as part of the overall testing program for environmental hazards. The use of an early biological end-point that can be shown to be related as a potential precursor to cancer is the first in vivo assay that seems rational. Although the assay is in a preliminary stage of development, it offers a novel approach to carcinogen testing that might well become valuable in a three tier system that is attractive - a mutagenesis test in a prokaryote as the first tier, a short term in vivo assay such as this one as the second tier and a long-term, whole animal test for cancer development as a third tier for those relatively few compounds that appear to be of great importance in industry or health.

Proposed Course: It is proposed to develop a sensitive radioimmunoassay for the PN-antigen as a possible useful index of the number of initiated hepatocytes induced by carcinogens and as a possible early test for preneoplasia.

It is also proposed to test in a systematic manner a series of polycyclic aromatic hydrocarbons, nitrosamines and nitrosamides, nitrofurans and nitroquinolines, as well as other types of carcinogens and a number of noncarcinogens in the new assay system. Also, it is proposed to continue to look for a substitute for 2-acetylaminofluorene as an important component of the selection system for resistant and putative initiated cells. In addition, it is planned to begin to explore the possible use of mice in place of rats from both economic and genetic viewpoints.

Date Contract Initiated: February 1, 1977

Current Annual Level: $92,613

UTAH, UNIVERSITY OF (NO1-CP-55709)

Title: Quantitation of Physiologic Reflux in Pancreatic Duct of Primates

Contractor's Project Director: Dr. Frank G. Moody

Project Officer: Dr. Carl E. Smith

Objectives: A primate model for the study of biliary pancreatic reflux under relatively physiologic conditions has been developed. Cannulae are inserted into the gallbladder and common bile duct of Rhesus monkeys and a pedicled segment of small bowel is used to create a pancreatic-cutaneous fistula after resection of the spleen and pancreatic tail. Following recovery, Hypaque is instilled into the gallbladder and its movement followed fluoroscopically. Simultaneous pancreatic fistula samples are obtained and assayed for Iodine by fluorescent excitation. Further studies involved the instillation of PEG-C^{14} into the gallbladder with subsequent sampling of fistula drainage by a flush technique. In an attempt to improve sensitivity, the model has been modified to include 80% pancreatic resection prior to creation of the fistula.

In conjunction with the primate reflux studies, a second phase of investigation has involved evaluation of the physiology and pharmacology of the sphincter of Oddi in the Opossum. The opossum provides an ideal animal model in that its easily accessible extraduodenal sphincter exhibits spontaneous electrical activity and simultaneous rhythmic contractions. Under light barbiturate anesthesia combined with skeletal muscle paralysis and mechanical ventilation, an array of 5 monopolar extracellular electrodes are placed along the sphincteric smooth muscle and contiguous duodenum. A catheter in continuity with a pressure transducer, drop counter, and saline reservoir is placed in the CD for simultaneous measurement of ductal pressure and flow. A second catheter is placed in the gallbladder for pressure measurement. Both the cystic and proximal common ducts are then ligated to isolate the CD from the GB and hepatic ducts. Pressure, flow, and electrical activity are then recorded simultaneously before and after the administration of test hormones or drugs. An identical experimental set-up has been used in several monkeys prior to sacrifice.

Major Findings: Reflux Study - The pancreatic duct was visualized in 21 of 34 radiographic studies (19 monkeys). Small amounts of Iodine were detected in the fistula effluent of 9 of 11 animals that refluxed radiographically. Radioactive polyethylene glycol (PEG-C^{14}) was instilled into the gallbladder and pancreatic fistula drainage sampled by aspiration (26 studies, 4 monkeys). When compared to controls, there was a significant rise in fistula counts beginning 50 minutes after injection and peaking at 180 minutes. In a second series of studies, pancreatic fistula aspiration was replaced by a flush technique using a triple lumen cannula which allowed constant monitoring and control of fistula pressure. A statistically significant rise and fall of radioactivity after PEG introduction was again demonstrated. In the animals that underwent 80% pancreatectomy prior to creation of the pancreatic fistula, one revealed no evidence of reflux by radiographic or isotopic criteria and the other demonstrated striking radiographic reflux, iodine retrieval, and PEG-C^{14} recovery.

Biliary sphincter study - The electrical activity of the opossum sphicter of Oddi precedes its mechanical activity and correlates directly with it. This is manifested by decreased ductal flow, increased ductal pressure, and increased frequency of bursts of spike potentials at the time of visible papillary muscle contraction. This electrical pattern is distinct from duodenal activity. Cholecystokinin, CCK-octapeptide (Kinevac), and pentagastrin all effect a nearly simultaneous significant ($p<.005$) increase in sphincter electrical activity and CD pressure with a decrease in CD flow. This action is independent of gallbladder contraction. Secretin has little effect on biliary kinetics. Dose-response studies with CCK, octapeptide, and pentagastrin show a direct relationship between dose and response with sphincteric contractile activity at all dose levels. Early studies have revealed a similar pharmacologic response to CCK and pentagastrin in the monkey biliary system.

Significance to Biomedical Research and the Program of the Institute: The primate model for biliary pancreatic reflux demonstrated unequivocal radiographic evidence of reflux of contrast material into the head of the pancreas, with only a modest physiologic rise in CD pressure during injection. The 62% incidence of reflux is in the range of the reported frequency of 7 - 46% during t-tube cholangiography in man. These studies also demonstrate that polyethylene glycol - C^{14} serving as a marker does pass into the pancreatic duct in a quantitative fashion. Thus, both radiographic and PEG reflux data demonstrate that biliary pancreatic reflux occurs frequently under these relatively physiologic conditions. This observation has not been previously demonstrated experimentally. While it does not directly shed light on the etiology of pancreatitis, it does lend credibility to the concept that bile born carcinogens might gain ready access to the proximal pancreatic duct.

Opossum data supports recent studies which refute the classical concept that CCK relaxes the sphincter of Oddi while causing the gallbladder to contract. It also clearly demonstrates the contractile effect of pentagastrin on the distal biliary sphincter, suggesting that they may act in harmony to modulate bile flow.

Proposed Course: The primate reflux model will be combined with the electrophysiologic and manometric study of the opossum and monkey sphincter of Oddi. This will allow a unique and comprehensive evaluation of the physiology and pathology of biliary kinetics. The immediate specific goals include: the investigation of the relationship between agents which clearly contract the sphincter (i.e., CCK and pentagastrin) and the frequency of biliary pancreatic reflux; investigation of the effect of prostaglandins on the sphincter of Oddi; investigation of a neurally mediated reflex between gallbladder and sphincter activity.

Date Contract Initiated: June 29, 1975

Current Annual Level: 0

VERMONT, UNIVERSITY OF (N01-CP-33360)

Title: Studies of Carcinogenesis in Organ Culture of Trachea and Bronchi

Contractor's Project Director: Dr. John E. Craighead

Project Officer (NCI): Dr. Michael B. Sporn

Objectives: This project has three basic objectives: 1) the development of organ culture systems which permit viability and differentiation of hamster tracheal epithelium for extended periods of time; 2) the development of techniques for the application of carcinogens quantitatively to respiratory tract epithelium in organ culture; and 3) the development of methods for the transplantation of organ culture tissue into susceptible hosts to document in vivo neoplastic transformation due to carcinogen exposure in vitro.

Major Findings: The goal of this project is the usage of tracheal organ cultures as an experimental model for the comparative morphologic assessment of the effects of respiratory carcinogens and cocarcinogens on the tracheal epithelium. As the progression of preneoplastic changes to frank neoplasia is thought to be a protracted process, a necessary prerequisite of these studies was the maintenance of the differentiated epithelium for extended periods of time in vitro. During the period of funding by this contract, techniques were developed for organ culture of the respiratory mucosa for as long as six months. The nutritional requirements of the differentiated and squamous metaplastic epithelium were explored in vitro. Subsequently, the polycyclic hydrocarbon, 3-methylcholanthrene (3 MC) was applied to the epithelium of explants in culture using synthetic fibers as carriers. Alterations in cellular morphology and multiplication resulting from exposure of the epithelium in vitro to standardized doses of carcinogen were documented.

Initial experiments showed that radiolabeled polycyclic hydrocarbons could be bound by low temperature precipitation to inorganic particulates such as ferric oxide (Fe_2O_3) and crocidolite asbestos. Ultrastructural studies

in this laboratory had shown that these dusts were either phagocytized or encompassed by the tracheal epithelium in vitro. In comparative studies, ^{14}C-3 MC was bound to Fe_2O_3 and crocidolite before their addition to cultures. Known amounts of particulates and carcinogen-particulate aggregates were then suspended in culture medium over a range of concentrations and precipitated on the epithelium of tracheal explants for one hour. Explants were maintained for periods of up to six months in culture before implantation into syngeneic hamsters. Carcinomas appeared with regularity in these studies, although fibrosarcomatous elements sometimes were present. Using crocidolite, it was possible to induce tumors with lower amounts of 3 MC. Comparative experiments with kaolinite and carbon as carriers of carcinogens are in progress.

The appearance of basal cell hyperplasia and squamous metaplasia in hamster tracheal organ cultures after addition of crocidolite of amosite asbestos was documented. In a recent study, the effects of the vitamin-A analog, retinyl methyl ether (RME) on these proliferative alterations were explored. Control explants and those exposed to asbestos were maintained in medium with and without addition of RME at 10^{-7}, 10^{-8}, and 10^{-9} M. At weekly intervals, alternate 5μ paraffin-embedded sections were prepared from cultures for autoradiography and histological assessment of metaplasia. In comparison to control cultures and those with addition of crocidolite, incorporation of tritiated thymidine was enhanced in organ cultures exposed to amosite asbestos ($p < .05$). Metaplastic changes in the epithelium were increased in explants exposed to both types of asbestos ($p < .05$). RME inhibited augmented DNA synthesis ($p < .01$) and metaplastic changes ($p < .001$) in a dosage-dependent fashion.

Significance to Biomedical Research and the Program of the Institute: The association of bronchogenic carcinoma with the polycyclic hydrocarbons, 3 MC and BP, is well documented in experiments administering these agents to laboratory animals. In vivo studies have indicated that particulates (i.e., carbon, Fe_2O_3) not only act as "carriers" of these carcinogens, but play an important role in tumor promotion. The reason for this tumor enhancement has not been determined. Organ culture of respiratory tract tissue affords a means for studying the interactions of carcinogens and foreign bodies with the differentiated epithelial cell.

Proposed Course: To conduct further studies in which quantitatively determined amounts of carcinogens are bound to particulates (carbon, kaolinite, asbestos, Fe_2O_3) or synthetic fibers that are administered to tracheal explants for predetermined periods of time in vitro. In parallel studies, intact segments of trachea will be removed from animals and these aggregates inserted into the lumen. After implantation into syngeneic animals, the appearance of tumors will be correlated with the amount and duration of carcinogen application to the mucosa. Attempts to develop an in vitro assay system for carcinogens will continue, and are intrinsic to this project.

Date Contract Initiated: June 28, 1973

Current Annual Level: $100,369

VETERANS ADMINISTRATION HOSPITAL (Tampa, Florida) Y01-CP-55625)

Title: Autoradiographic Study of the Cellular Response of the Respiratory Tract in Chemical Carcinogenesis

Contractor's Project Directors: Dr. Hollis G. Boren
Dr. Lois J. Paradise

Project Officer (NCI): Dr. Curtis C. Harris

Objectives: To determine the proliferative cellular response of segments of the respiratory tract following exposure to respiratory carcinogens and particulates, using the Syrian hamster. These measurements are correlated with the histopathologic response to carcinogens and particulates. Tritiated thymidine incorporated by respiratory epithelial cells is determined by quantitative light microscopic autoradiography. Serial sacrifice studies are performed to define the morphogenesis of squamous cell carcinoma of the lung.

Major Findings: The chemical carcinogen N-methyl-N-nitrosourea (MNU, 1% in 0.1M citrate buffer pH 5.7 containing 10% ethanol) was applied with an automatic cannula to prescribed areas of tracheal epithelium of Syrian hamsters once a week, one to 15 times. A series of experiments designed to measure changes in cell populations (i.e., cellular differentiation) and in proliferative cell cycles in tracheal epithelium are in progress. Findings to date include: 1) presence of squamous cell carcinomas in tracheas of all hamsters (14) killed 7 or 8 weeks after the last of 15 weekly exposures to MNU. There was also an adenocarcinoma in one hamster trachea. These neoplasms were all in those areas of tracheas which were washed with MNU solution; no tumors developed in the buffer-treated control hamsters. 2) The extent of changes in the tracheal epithelial cell population increased with increased number of applications of MNU. There was a rapid decrease in mucous cells with a more gradual reduction in ciliated cells. Undifferentiated "luminal" cells replaced these cell types. In hamsters given 3 or more applications of MNU, giant (nuclei = 20-50μm) basal, giant "luminal" and giant ciliated cells were observed and there were areas of squamous metaplasia. 3) For measurement of changes in cell kinetics, hamsters were injected intraperitoneally with [^{3}H]thymidine 5 times at 14 hour intervals starting 2 weeks after 1 and 5 applications of MNU or buffer. Fourteen hours is longer than the durations of DNA synthesis of basal and mucous cells (the proliferative compartments) in unstimulated hamsters tracheal epithelium. Thus 5 injections of [^{3}H]-thymidine should give about 5 times as many labeled cells as a single injection without labeling the same cells twice. Hamsters were sacrificed 7 and 19 days after the last [^{3}H]thymidine injection (24 and 36 days after the last MNU or buffer application). Changes in cell populations, labeling indexes, and normalized grain counts per cell were more enhanced after 5 exposures to MNU than after one exposure. The main changes observed after MNU 5 times were: a) increase in average size of cells of a given type; b) absence of normal cellular differentiation; c)

labeling indexes for the various kinds of cells 5-40 times higher after MNU than after buffer; d) mean normalized ^{3}H-grains/cell (NGC) indicating that some labeled cells either were not dividing or had greatly lengthened cycle times. In unstimulated hamster tracheal epithelium, basal cells and mucous cells are the proliferative compartments. In MNU-treated epithelium, normal epithelium, normal basal cells and "luminal" cells appeared to be the main proliferative compartments since after 5 exposures to MNU, NGC at 19 days were significantly less than NGC at 7 days, indicating that the cells were dividing. In giant basal, giant ciliated, giant "luminal" cells, squamous, suprabasal and ciliated cells, NGC did not decrease significantly from 7 to 19 days after [^{3}H]thymidine suggesting that [H]thymidine was incorporated before the cells differentiated and not thereafter. Although these new cell types appear after MNU-treatment, they seem unlikely to be precursors of neoplasms if they prove to be nonproliferating by other cell kinetics methods currently being employed.

Significance to Biomedical Research and the Program of the Institute: This contract is an integral part of an interlocking group of contracts designed to define the roles of carcinogens and the physicochemical factors required for respiratory carcinogenesis. The MNU tumorigenesis experiment confirms results of other investigatiors using this method of application of this carcinogen while it demonstrates the validity of using changes in cell kinetics in tracheal epithelium exposed to chemical carcinogen as indicators of still broader biologic effects of carcinogens.

Proposed Course: A combination of repeated labeling, double labeling with [^{3}H]-and [^{14}C] thymidine and normalized grain count methods is being used to study cytokinetics of hamster tracheal epithelium exposed to *N*-methyl-*N*-nitrosourea in limited areas. Alterations will be correlated with histologic changes and time of appearance of tumors.

Date Contract Initiated: June 2, 1972.

Current Annual Level: $106,784

VETERANS ADMINISTRATION HOSPITAL (Washington, D.C.) (Y01-CP-60204)

Title: Studies on Normal, Premalignant, and Malignant Respiratory Epithelium of Humans

Contractor's Project Director: Dr. Paul W. Schafer

Project Officer (NCI): Dr. Curtis C. Harris

Objectives: 1) To affect a better morphologic and biochemical characterization of normal, premalignant and malignant respiratory epithelium; 2) to obtain and to establish organ cultures of essentially normal human lung tissues so that they may be studied in their response to carcinonges both in culture and by xenotransplantation into immune deficient experimental animals; 3) to study the effects of chemotherapeutic or antineo-

plastic agents on evolving premalignant or in-situ malignant changes induced in experimental animals, by cytochemical, light and electron microscopic technics; and 4) to structure a lung cancer classification based on coordinated input from all collaborators.

Major Findings: Segmental and lobar bronchi together with substantial amounts of pulmonary parenchyma have been obtained from forty-three patients undergoing the following operations; 10 pneumonectomies, 28 lobectomies, 2 bi-lobectomies and 3 segmental resections. A detailed abstract of each patient's clinical record has been prepared. Gross pathology encountered at surgery together with pertinent aspects of the operative procedure have been entered in the input protocol. Only those tissues were resected that were required for diagnostic and therapeutic purposes. With a tissue proven preoperative diagnosis a definitive resection without further biopsy was performed when technically possible. In the absence of a positive preoperative diagnosis, the diagnostic procedure of choice has been total local excision; and only when this was not technically possible was incisional biopsy used. No tissues were obtained solely for the purpose of this investigation.

All resected specimens have been aseptically dissected promptly upon removal from the patient. Parts of the specimen critical to the requirements of the WVAH Laboratory Service, particularly those tissues at the line of resection, have been left untampered. As much of the bronchial tree as otherwise could be obtained has been dissected free. Adequate samples were taken of all disease processes encountered. Representative samples of the foregoing have been variously prepared for light and electron microscopy and the remainder have been placed in L-15 medium and stored at 4°C for subsequent cultural and xenotransplantation studies.

By light microscopy it has been ascertained that three of the patients did not harbor a malignancy but rather had bronchiectasis, sequestration and cryptococcosis respectively. The forty malignancies have been variously identified as follows: 22 squamous cell, 6 adeno, 5 undifferentiated, 3 broncho-alveolar, 3 carcinoid and 1 in-situ. In addition gastric and esophageal mucosa were obtained from a specimen resected for esophageal carcinoma. All of the foregoing materials were prepared for ultramicrotomy and electron microscopy and will be so characterized along with high resolution light microscopy. Most of these recovered tissues have been successfully propagated in culture.

Significance to Biomedical Research and the Program of the Institute: Model systems such as are employed in this collaboration incorporating human tissues either in culture or in experimental hosts should be promising targets for experimental carcinogenesis by suspect classes of environmental agents. Ultimately they may come to represent the basis for development of new prophylactic and definitive therapies.

Proposed Course: 1) Continue obtainment of viable normal and abnormal human pulmonary tissues under those strict medical, legal and ethical strictures which characterize human experimentation; 2) Visible light and

electron microscopic characterization of the foregoing; and 3) Enlarged collaboration in the maintenance of these tissues in vitro, their xeno-transplantation into immune deficient animals and their experimental treatment in both settings.

Date Contract Initiated: March 1, 1976

Current Annual Level: $29,138

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY (NO1-CP-55685)

Title: Relationship of Fecal Neutral Sterols to Large Bowel Colon Cancer Risk

Contractor's Project Director: Dr. Tracy D. Wilkins

Project Officer (NCI): Dr. Carl E. Smith

Objectives: To continue studies on factors affecting the metabolism of neutral steroids in the colon, including the presence of fiber and mucin. To investigate and determine properties of ether-extractable mutagens detected in human fecal samples by the Ames test and the possible relationship of these substances to fecal lipids and colon cancer.

Major Findings: A major finding in the past several months has been that mutagens, extracted from feces with ether, are more prevalent in populations at high risk for colon cancer than in populations at low risk. Contractor tested feces from two high-risk populations (55 North Americans and 42 urban, white South Africans) and two low-risk populations (108 rural and 65 urban, black South Africans) have been tested using the Salmonella/ mammalian microsome mutagenicity test of B. Ames. Ten percent of the North Americans and 19% of the urban, white South Africans excreted significant amounts of mutagenic substances, whereas none of the rural black and only 2% of the urban, black South Africans did. The difference between high- and low-risk populations was staistically significant at the p = 0.001 level. The ether extraction procedure used to remove neutral steroids from human feces will also extract the mutagenic substances, which were detected using Ames' test. Since mutagens may be carcinogenic, it may be possible that mutagenic substances extracted from feces may be involved in colon cancer.

Studies are presently underway to determine if excretion of mutagenic substances are correlated with neutral steroid excretion patterns.

Mutagen excretion has also been monitored in six individuals over the past several months. It appears that individuals originally donating fecal samples which contained mutagens continued to excrete mutagens in 12-65% of their fecal samples. Individuals originally designated as non-excreters have never provided us with a mutagenic fecal sample.

Studies on fecal mutagens to investigate their production and inactivation have found that the mutagenic capacity of fecal extracts can be altered by colon bacteria, mammalian enzymes, and by fiber and mucin. The usual effect is reduction of mutagenicity, although colon bacteria have activated some fecal extracts and inactivated others.

Significance to Biomedical Research and the Program of the Institute: Mutagenic substances found in human feces could prove to be carcinogens. Since neutral steroids may be involved, it is important to determine how components of the colon contents, including anaerobic bacteria, interact with neutral steroids and the possible relationship of fecal steroids, mutagens, bacteria, and colon cancer risk.

Proposed Course: Continue studies on the production and inactivation of fecal mutagens by colon bacteria. Correlate excretion of fecal mutageans with patterns of neutral steroid excretion in populations at different risk for colon cancer. Populations from which samples are presently being collected include Japanese, Scandinavian, and colon cancer patients.

Date Contract Initiated: June 1, 1975

Current Annual Level: $137,000

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY (N01-CP-33334)

Title: Comparative Fecal Flora Studies

Contractor's Project Director: Dr. W. E. C. Moore
Dr. L. V. Holdeman

Project Officer (NCI): Dr. Carl E. Smith

Objectives: To define and compare statistically the bacterial flora of the feces and colon of human populations with different risks of colon cancer.

Major Findings: Procedures used in this study give the highest cultural recovery of intestinal and fecal bacteria yet reported. Cultural recovery is up to 95% of the direct microscopic count of 200 to 400 billion bacteria per gram. Viable bacteria constitute about 40% of the fecal volume. Statistically randomized sampling of isolated colonies and analysis of 100 or more properties of the isolates determine the relative concentrations of the kinds of bacteria that each represent one-thousandth of the flora or more. Studies of a control population and of daily variation in bacterial composition of the feces have been completed and published, together with descriptions of 24 new species that predominate but which had not been detected or recognized by other workers. It also has been necessary to publish emended descriptions of several other species. Data and isolates from three additional studies are now being analyzed. These include: 1) polyp patients vs. rural Africans and Japanese; 2) the entire

tract of 6 cadavers taken within 2 to 4 hours of accidental death (showing that the fecal flora is representative of the colonic flora, and that the major intestinal bacterial growth occurs in the cecum and ascending colon, a frequent site of colon cancer); and 3) fecal floras of 5 people on all rice, then all beef, then all leafy vegetable diets. The major technical difficulty results from the lack of previous information on most of the 350 bacterial species encountered among the 11,000 isolates from these studies. J. L. Johnson of this laboratory has demonstrated by DNA-DNA homology experiments that differentiation, even of common Bacteroides species, has been inaccurate by conventional procedures. Because these species predominate but differ in bile metabolism (possibly important in carcinogenesis), the last year has been spent in reanalyzing over 2,000 bacteroides isolates from the populations being studied. It has been found that B. uniformis (formerly confused with B. thetaiotaomicron by this and by other laboratories) is the most sensitive to physiological levels and effects of epinephrine (probably including bile secretion) in response to anger or fear (sig. at 0.0003 level). Preliminary analyses suggest that there are some statistical differences in flora composition that correlate with risk of colon cancer. One group of bacteria (found to include 3 genetic species) has a negative correlation with risk. Comparison of the two low risk groups with high risk and medium risk groups, did not reveal the bacterial differences reported by previous workers. J.L. Johnson has shown that isolates of the bacterial species in this laboratory are genetically the same from each of the different human populations. The data suggest that incomplete analyses and the use of API test strips that are inadequate for accurate identification of fecal bacteria probably account for discrepancies among results from different investigators in this field.

Significance to Biomedical Research and the Program of the Institute: Based on the identification systems developed, several matching sets of bacterial strains have been provided to other laboratories who are investigating specific metabolic activities that may be related to the production or metabolism of carcinogenic compounds. The relative numbers of these bacteria have been documented in human populations having different risks of colon cancer. Recent research by others demonstrates differences among human populations in levels of fecal mutagens or potential carcinogens. If this or other new information indicates a relationship between intestinal metabolism and carcinogenesis, identification of the specific agents responsible for the processes involved will require reliable differentiation and recognition of the intestinal bacterial species. Procedures for accurate differentiation of the intestinal bacteria are being developed in the current project and this comparative analysis itself may provide leads concerning colon carcinogenesis and/or protection from colon cancer.

Proposed Course: Studies under this contract will continue to the planned expiration in June, 1978.

Date Contract Initiated: June 1, 1973

Current Annual Level: 0

W. ALTON JONES CELL SCIENCE CENTER (N01-CP-65762)

Title: In Vitro Cultivation of Normal, Prostatic Epithelial Cells

Contractor's Project Director: Dr. William H. J. Douglas

Project Officer (NCI): Dr. Stuart H. Yuspa

Objectives: 1) Establish primary cultures from the ventral lobe of rat prostate. 2) Characterize these primary cultures to determine the specific properties of normal prostatic epithelium that are retained in vitro.

Major Findings: A method was developed for establishing primary cultures of prostatic epithelial cells. Rat ventral prostate was enzymatically dissociated in 0.5% collagenase in F12K medium supplemented with 1% fetal bovine serum. The resulting primary cell suspension contained several populations of cells and was enriched for the epithelial cells by employing selective attachment techniques. The majority of fibroblasts attached to the culture vessels during the first 48 hours in vitro. Epithelial cells, however, did not attach at this time and were readily removed from the adherent fibroblasts by a medium change. When these epithelial cells were plated into new vessels, they attached within 18 to 24 hours and colonies of epithelial cells were generated. Epithelial cells incorporated tritiated thymidine during this time period as demonstrated by cell culture autoradiography.

Electron microscopic examination of the epithelial cell colonies demonstrated that the cells were morphologically similar to prostatic epithelial cells in vivo. Cells comprising the epithelial colonies contained numerous secretory vesicles and tonofilaments with adjacent cells joined by tight junctions and desmosomes.

In addition, procedures were developed for histochemical localization enzymes and specific staining techniques that can be used as markers for rat ventral prostatic epithelial cells. In order for a marker to be classified as specific for prostate epithelium one must be able to demonstrate its specificity for acinar epithelial cells in 10μm cryostat sections of rat ventral prostate. Once this was accomplished the usefulness of that marker on prostatic epithelial cell cultures was evaluated.

With these enzyme localizations and staining reactions one can demonstrate that the primary cultures of epithelial cells retain prostatic-specific functions, in vitro. Cultures that consist entirely of prostatic fibroblasts did not retain any of these epithelial cell-specific functions, but they did synthesize collagen in vitro.

The data available to date indicated that after one week in vitro the primary cultures were comprised primarily of epithelial cells of prostatic origin and that only a few contaminating fibroblasts were present.

Significance to Biomedical Research and the Program of the Institute: Among neoplastic diseases in the United States, prostatic adenocarcinoma is the third most common cause of death in adult males. The study of the early cellular changes that occur prior to the onset of human prostatic adenocarcinoma has been hampered by two factors. Human tissue, obtained at autopsy or from diagnostic biopsy, is difficult to obtain in a disease-free state. Spontaneous tumors rarely occur in laboratory animals, and attempts to induce tumors resembling the human cancer in these animals have not been successful. The availability of an in vitro model system for growth of normal prostatic epithelium would facilitate the study of these preneoplastic changes.

Proposed Course: During the next contract period, improvements will continue to be made in the cell isolation procedure to further enrich the primary cultures for epithelial cells. In addition, the efficacy of a variety of hormones, vitamins and other growth factors for supporting optimal growth of the prostatic epithelial cells will be evaluated. Several biochemical assays (androgen-receptor binding and epithelial cell-specific enzyme assay) will be utilized to further characterize the epithelial cultures. These later studies will aid in quantification of the effects of various media supplements (hormones, vitamins, growth factors) on the primary cultures.

Date Contract Initiated: September 30, 1976

Current Annual Level: $120,800

WISCONSIN, UNIVERSITY OF (NO1-CP-75909)

Title: Long-Term Studies of Prevention of Epithelial Cancer by Retinoids

Contractor's Project Directors: Dr. George T. Bryan
Dr. William Croft

Project Officer (NCI): Dr. Morton H. Levitt
Dr. Carl E. Smith
Dr. Michael B. Sporn

Objectives: The object of this program is to acquire information concerning the effect of synthetic retinoids on the development of urinary bladder cancer in experimental animals. The present study is aimed at obtaining unequivocal data for the prevention of bladder cancer during the neoplastic period by different retinoic compounds in rats. An experimental model for the induction of rat bladder cancer by N-[4-(5-nitro-2-furyl)-2-thiazolyl]-formamide (FANFT) has been developed in this laboratory. This model will provide a good oportunity for the determination of the efficacy of retinoid compounds in the prevention of bladder cancer during the preneoplastic period.

Major Findings: Since January 9, 1978, 965 rats of ten different groups were placed on study using FANFT as the carcinogen, and 13-cis retinoic acid as the retinoid. Placebo is used as carrier of the retinoid. To date, the 13th week of the study, no gross evidence of tumors has been detected, but the animals are scheduled to go for 50 weeks. At the end of the study, each rat will be evaluated for bladder weight, number of tumors per badder detected grossly, percent urinary bladder having hyperplasia, papilloma, and carcinoma. Histological evaluation of each lesion will be based on the classification of the World Health Organization.

Significance to Biomedical Research and the Program of the Institute: The literature has demonstrated potential use of retinoids in inhibition of cancer in man and animals. This study will provide data concerning the efficacy of 13-cis retinoic acid in the inhibition of urinary bladder cancer induced by FANFT. Retinoids, then, if found to be effective in such studies, could be used in the chemoprevention program aimed at inhibiting the formation of urinary bladder cancer in the preneoplastic stage and reducing the incidence of bladder cancer in man until better modes of prevention or therapy can be established.

Proposed Course: Pursue similar studies of inhibition of bladder carcinogenesis using other retinoids and refinements in the FANFT model.

Date Contract Initiated: September 30, 1977

Current Annual Level: $212,000 (Supported by Carcinogenesis Testing Program)

SUMMARY REPORT

CHEMISTRY OPERATIONAL UNIT

October 1, 1977 through September 30, 1978

The Chemistry Operational Unit provides administrative and program management direction for two complementing Collaborative Research Segments, the Carcinogen Metabolism and Toxicology Segment, and the Chemistry and Molecular Carcinogenesis Segment. The Carcinogen Metabolism and Toxicology Segment has as one of its goals the identification of those components of complex systems which function as environmental carcinogens and the development of analytical methodology for their detection and quantitation. Metabolic and toxicologic studies of environmental carcinogens in selected animal systems are conducted to provide additional knowledge on the metabolic pathways involved in the carcinogenic process. Such studies may also furnish an explanation for the varying responses of different animal species and man to chemical carcinogens. The mechanism of chemical carcinogenesis in selected animal systems with defined characteristics is being investigated. The major objective of the Chemistry and Molecular Carcinogenesis Segment is to gain an understanding of the molecular process involved in chemical carcinogenesis and an understanding in chemical biological terms of the important events that lead to carcinogenesis.

Studies in the Metabolism and Toxicology Segment have been grouped into several major approach areas: (1) metabolism and mechanism of carcinogenesis; (2) identification of environmental carcinogens and (3) effects on chemical carcinogenesis. The major accomplishments of these approaches in the last year are highlighted below:

Metabolism and Mechanism of Carcinogenesis From Southern Research Institute (NO1-CP-55721) it was reported that 4-chloro-2-methlyaniline binds appreciably to rat liver protein, DNA, and RNA. A microsomal metabolite was 4,4'-dichloro-2-dimethylazobenezene implying that an intermediate probably was 4-chloro-2-hydroxylaminotoluene. In addition bromoacetaldehyde has been positively identified as a microsomal metabolite of 1,2-dibromoethane. 1,2-Dibromoethane did not bind to synthetic polynucleotides in vitro but only after microsomal activation.

Many different urinary metabolites of Michler's ketone have been identified in studies on this compound. The proportion of Michler's ketone bound to DNA was the same as that of the known carcinogen, N-2-fluorenylacetamide (Pacific Northwest Research Foundation, NO1-CP-55719).

In the metabolism of the industrial intermediate o-toluidine, many urinary metabolites, both conjugated and unconjugated, were identified. Of all these metabolites only 2-nitrosotoluene and N-2-methylphenylhydroxylamine were found to be mutagenic. There was a direct linear relationship between the amount of excreted 2-nitrosotoluene and the administered

o-toluidine. From the mutagenicity studies it seems that the o-methyl group enhanced the effect similar to the in vitro results where o-methylation of aromatic amines enhances the carcinogenic activity (American Health Foundation; NO1-CP-55639).

Using the pulmonary tumor induction technique in strain A mice it was found that 4 selected organo halides, 4-chloro-1-butanol, 3-chloro-propionic acid, 2-bromoethanol, and 2-chloro-N,N-dimethylethylamine hydrochloride showed tumorigenic activity. These compounds contained a nucleophilic substituent in addition to the halide moiety indicating that nucleophilic substitution increases the tumorigenic effect. It was found that injection of caffeine suppressed both the spontaneous and urethan induced pulmonary tumors in strain A mice. Mechanistic studies showed that this was probably due to general suppression of lung DNA synthetic activity (University of California, San Diego, NO1-CP-33232).

Identification of Environmental Carcinogens In this area are various projects such as those at the University of Hawaii (NO1-CP-75915). A procedure has been developed under this contract to detect trace quantities of cycasin and macrozamia after addition to meat. There are many interfering compounds present in meat which has hindered development of the technique but a clean-up procedure has been developed. The analytical technique is thus capable of detecting minute quantities of these derivatives of methylazoxymethanol.

In another contract at Midwest Research Institute (NO1-CP-23270), considerable effort has been expended in identifying the metabolic products of 3-methylcholanthrene. These studies indicated that liver and pancreas extracts formed the 1-hydroxy derivative of 3-methylcholanthrene and that a cis-1,2-diol was present in the extract from pancreas studies. Metabolism at the 11,12 position also occurred as indicated by the presence of the 11-hydroxy-, 11,12-quinone and the cis-11,12-diol from 3-methylcholanthrene. However, the quantity was much less than that of the metabolites of the 1-position.

Several projects both in the analytical and in the mechanistic areas deal with nitrosamines as environmental carcinogens. A contract with the British Food Manufacturing Industries Research Association (NO1-CP-43337) has led to a method to determine nitrosamine compounds using a chemiluminescent analyzer. Reproducibility studies on low levels of N-nitrososarocosine as a representative nonvolatile nitrosamine have been conducted. In addition procedures have been developed whereby many nitrogenous compounds other than nitrosamines and nitrosamides can be differentiated from the N-nitroso compounds.

A study at the University of Missouri (NO1-CP-75946) showed that the compound bis-2-hydroxyethylnitrosamine which contaminates certain cutting fluids can be fragmented to dimethylnitrosamine and 2-hydroxyethylmethylnitrosamine when treated with potassium tertiary butoxide at 70°C. The yield of each product is approximately 10% after 48 hours. In addition, some other very active nitrosamines such as nitrosomorpholine,

2-hydroxyethylvinylnitrosamine and methylvinylnitrosamine were formed as well, some in relatively high yields. Treatment of nitrosomorpholine with strong base converted this compound to dimethylnitrosamine and 2-hydroxyethylmethylnitrosamine as well as other compounds. In view of the large number of people exposed to cutting fluids in metal machining operations, these results indicate that a hazard may exist since temperatures probably exceed 70° without great difficulty at the metal-tool interface.

Another project in this area is that with the Massachusetts Institute of Technology (N01-CP-33315). This project has concentrated on the etiology of gastric cancer in a specific population in Columbia, South America. It was found that gastric samples which had a high pH were also very high in nitrite when nitrate was available. In view of the nitrate content of the vegetables and water in these regions, a partial explanation for the high gastric cancer incidence may be at hand. Nitrate metabolism in persons on defined diets which were low in nitrate showed that urinary excretion of nitrate was extremely variable but generally exceeded nitrate intake. This suggests that endogenous formation occurred. Further investigation of the phenomenon indicated that nitrite and nitrate may be formed in the intestines by heterotrophic nitrification of ammonia in the intestinal tract. Other results suggested that preformed nitrosoproline was not the major precursor of the nitrosopyrrolidone in the fried bacon. In addition, it was found that deliberate nitrosation of corn and other legumes from the high risk gastric area of Columbia yielded several compounds which gave a response on the thermal energy analyzer. However, all these compounds were not nitroso compounds; one in corn was nitrohexane. The significance of such compounds to gastric cancer is unknown.

Treatment of primary amines with nitrite leads to a certain degree of formation of dialkylnitrosamines, contrary to what was thought previously when nitrosation of primary amines generally led to alcohols.

Effects of Diet on Carcinogenesis One contract with MIT (N01-CP-33238) has determined that semi-synthetic diets which are high in fat and marginally deficient in choline, methionine and folic acid, can enhance induction of certain types of tumors from known carcinogens but may decrease induction of other types of tumors. Of interest was the fact that certain hepatic microsomal oxidases were decreased in animals on these diets. The clearance of certain nitrosamines was decreased, but that of others increased; the plasma content of alphafetoprotein, usually taken as an indicator of a neoplasm, was increased.

Another project related to dietary contaminants at MIT (N01-CP-43265) has led to characterization of the major DNA adduct of aflotoxin B, as 2,3-dihydro-2-(N7-guanyl)-3-hydroxy-aflotoxin B. Various other mycotoxins, isolated from organisms contaminating foodstuffs, were mutagenic in the Ames type Salmonella system.

Studies in the Chemistry and Molecular Carcinogenesis Segment have been grouped into the following five major approach areas: (1) biochemistry

of chromatin, nucleic acids and DNA repair; (2) study of surface membranes and the manner in which they participate in the regulation of cellular properties; (3) study of the genetics of somatic cells, the chemically-induced mutations that can be induced in them, and the significance of this process in carcinogenesis; (4) study of polycyclic hydrocarbons and the significance to carcinogenesis of the many products which result from their enzymatic activation; and (5) studies on the role of mutagenesis in carcinogenesis.

Biochemistry of Chromatin, Nucleic Acids and DNA Repair Studies at Vanderbilt University School of Medicine (N01-CP-65730) had shown earlier that certain non-histone DNA-proteins from the nucleus of malignant cells differed from similar proteins from normal cells. The immunological properties of these proteins have now permitted a histological examination of their cellular localization, both in normal cells and in the cells from which the proteins were derived. Antiserum from normal cells localized clearly in the nucleus. Similar localization was observed when antiserum to complexes derived from hepatoma were tested with hepatoma cells, but the hepatoma antiserum did not localize in normal rat liver tissue. The Novikoff hepatoma antiserum, however, did stain hepatocyte nuclei in regenerating liver in agreement with earlier findings showing that the regenerating hepatocyte contained nucleoprotein DNA complexes which reacted with the hepatoma antiserum. Extensions of this approach may be helpful in identifying malignant cells histologically. Additional studies from this laboratory on the possibility of separating individual protein constituents from the immunologically reactive group of non-histone proteins have provided encouraging results.

Investigators at the Weizmann Institute (N01-CP-33220) have conducted studies on the interaction between chemical carcinogens and other biologically active chemicals and viruses. The infectivity of SV40 DNA to which benzo[a]pyrene diolexpoxides were bound was significantly less effective in productive infection, although replication of reacted DNA was normal. In an interesting and possibly significant study these investigators have shown that some biochemical changes which occur subsequent to carcinogen treatment may be so subtle as to require extremely sensitive testing to be revealed. In their experiment, transfer RNAs were isolated from animals treated with ethionine. By all conventional methodology these tRNAs were not different from those obtained from normal animals, but when their function was assayed in vivo by microinjection into *Xenopus* oocyte cytoplasm, these tRNAs were found to be remarkably impaired in their capacity to sustain protein synthesis. Other studies from this laboratory have studied viral RNA synthesis, viral DNA synthesis and the production of C-type virus particles after treatment with chemical carcinogens.

Earlier studies at the Brookhaven National Laboratory (Y01-CP-50202) had shown that UV irradiated thyroid cells injected intraperitoneally into isogenic recipient fish resulted in thyroid tumors. The question of why thyroid tumors specifically were found has been resolved by labelling the membranes of irradiated cells with iodine-125. Radioautography showed

that these cells migrated to the thyroid and preferentially concentrated there. This contractor is now testing eight chemicals that mimic either UV action or ionizing radiation action on their effect on DNA for their quantitative effect in inducing thyroid tumors. Preliminary results have shown that one treatment with DMBA gives rise to thyroid tumors which will metastasize.

At the Oak Ridge National Laboratory (Y01-CP-50200) extensive studies on DNA damage and repair caused by safroles have indicated that damage to DNA by these agents is repaired by a heretofore undescribed DNA repair system, which is currently under investigation. Studies on a series of human colon carcinoma cultures have confirmed there is a reduction of 50-70% of normal repair as compared to normal human fibroblasts.

Surface Membranes Investigations at the Johns Hopkins University (N01-CP-45610) have further defined and extended the observations reported last year on calcium transport by tumor mitochondria and the role of protons in the generation of ATP. They have found that the relative rates of calcium influx and efflux in tumor mitochondria are independently regulated by changes in the oxidation reduction state of mitochondrial pyridine nucleotide co-enzymes. A shift to a more reduced state depresses the rate of calcium efflux. It has been established that four protons are ejected per pair of electrons per site by the energy transducing electron carriers in the mitochondrial membrane and that three are ejected per ATP hydrolyzed while four must pass into mitochondria for each ATP molecule synthesized. These values have been observed in mitochondria from normal rat liver, heart and brain and Ehrlich tumor cells. They have also identified a second major action of calcium within the mitochondria, namely, that it inhibits ATP synthetase directly. Thus, the penetration of calcium into the mitochondrion at the expense of ATP and subsequent inhibition of ATP synthesis may signal an increased rate of glucose utilization by the cell.

At the University of Rochester (N01-CP-45611) further study has indicated that the elevations in phospholipid found in transformed cells is due to a newly described component, phosphatidyl threonine. Normal embryonic and transformed cells of the mouse and chick did not show such an elevation, suggesting that it is a species-specific response of the hamster cell to altered growth control. A variety of nucleotide amino acids and benzamidine derivatives have been synthesized and their basic photochemistry, an ability to inhibit the activity of enzymes, determined.

Investigations at the Hebrew University (N01-CP-43307) have led to two conclusions: 1) that transport is not affected when growing and non-growing, and normal and malignant cells are compared, at least as far as the uptake of uridine is concerned; 2) it is the trapping of uridine by phosphorylation that is enhanced when non-growing cells are stimulated to grow and this control is lost when cells are transformed. It has been found that numerous inhibitors of uridine uptake affect equally both transport and metabolic trapping.

At the Oak Ridge National Laboratory (Y01-CP-50200) studies on amino acid uptake by cells have indicated that this transport is coupled to sodium ion movement. A major factor in this transport is the electrical potential across the membrane. In these experiments BHK cells that had been transformed into temperature-sensitive mutants by chemical carcinogens were used. The transport rates and membrane potentials of these cells increased when they were growing at their transformed temperature. Promoting agents have been found to increase the potential and it has been provisionally concluded that the membrane potential may be a causal factor in growth regulation. Other studies have examined the uptake and metabolism of glucose. It was found that hexokinase is membrane-bound in the transformed cells and that during induction of enhanced glucose transport there is co-induced a ten-fold increase in glucose-6-phosphatase activity.

Somatic Cell Genetics Work at the University of Ontario (N01-CP-43331) on mutagenesis has provided further indications that the ability to obtain recessive mutations in CHO cells may be due to functional hemizygosity for the genes in which the mutations have been observed. This laboratory has developed methods for chromosome and DNA transfer in CHO and other cell lines. The nature of the process and the resulting genetic properties have been characterized. These new methods have permitted experiments leading to the conclusion that carcinogenesis in hamster cells in vitro occurs in two distinct steps. In the first step, normal cells are converted into morphologically transformed cells. This is followed by a second step in which the above colonies can be converted into tumor-producing cells by transfer of a second chromosome. These studies may have importance in the question of aging as well as malignancy.

At New York University School of Medicine (N01-CP-33279) interesting new mutants of a hamster cell line BHK21 have been generated. Among those are cell lines mutant due to the presence of a temperature-sensitive ribosomal protein. Of particular interest are a group of mutants which at 39° become arrested in the G1 phase of the cell cycle. These several mutants differ from each other and have been used to study the factors required for the exit from a resting state in BHK cells. Malignant cells differ from normal cells with respect to exit from G1 and these mutants may prove valuable in understanding the nature of this step.

Investigators at Yale University (N01-CP-55673) have found that new combinations of F_1 animals between inbred strains of mice will yield increased teratoma cell populations with near diploid chromosome constitutions. These lines can be used as sources for monosomic cell populations. Cell hybrid and microcell hybrid experiments have enabled the mapping of eleven new gene loci.

Encouraging progress is being made in attempts to activate the proliferation of spermatids and sperm cells into haploid cell lines. Treatment of spermatids with concanavalin A has been observed to stabilize the cells prior to subsequent treatment with polyethylene gycol for fusion of the sperm cells with red blood cell ghosts which have been loaded with egg cytoplasm.

At the University of California at San Francisco (N01-CP-43239) mutants have been isolated whose primary defect is an 80% reduction in the level of adenylosuccinate synthetase activity. Other clones with altered levels of enzymes involved in synthesis of pyrimidine nucleotides have been isolated and characterized with respect to their enzyme levels. These mutants have been used to advantage in understanding the normal regulation of pyrimidine nucleotide synthesis as well as metabolism of the clinically useful cancer therapeutic drug, 5-fluorouracil. Other mutants have indicated that aza-substituted nucleosides are transported by cellular mechanisms genetically distinct from those that transport naturally occurring nucleosides.

Polycyclic Hydrocarbons Results from three contracts dealing with the induction of aryl hydrocarbon hydroxylase in human lymphocytes are conflicting. At the University of Wisconsin (N01-CP-65734) an excellent correlation has been found between the metabolic rate for benzo(a)pyrene in mitogen-stimulated lymphocytes and the half-life of anti-pyrene in vivo. Lung cancer patients had significantly higher oxidation rates for anti-pyrene compared to matched controls. These results are in confirmation of earlier results from this group showing that persons with genetically controlled increased enzymatic induction are at greater risk to lung cancer than normal subjects.

On the other hand, at Health Research, Inc., Roswell Park Memorial Institute (N01-CP-55629) further studies with lung cancer patients and matched controls failed to indicate a difference in AHH activity or inducibility between the two groups. This group also confirmed that the metabolism of anti-pyrene was correlated with the microsomal AHH and lymphocytes.

The University of Texas System Cancer Center (N01-CP-55604) did not observe the seasonal variation in AHH activity reported previously under contract N01-CP-55629 and further report the solution of a number of other technical difficulties with the assay. The group found significant differences between 600 cancer patients and 500 normal donors; patients with bronchogenic carcinoma and oralpharyngeal squamous carcinoma both had significantly higher AHH inducibilities than normal donors. Patients with other types of cancer did not differ from the normal population.

At the Oak Ridge National Laboratory (Y01-CP-50200) studies have been conducted characterizing the metabolic products derived by using as source of enzyme a number of rodent and human cells, and tissues. The use of microsomes as opposed to whole cells gives rise to significant differences in the number of derivatives as final products which are found. In addition, some epithelial cell lines have been studied in an attempt to identify the activation and detoxification pathways in a variety of cells.

Mutagenesis in Carcinogenesis Further progress in the study of the significance of mutagenesis in carcinogenesis have been reported during this year. At the Ontario Cancer Institute (N01-CP-43331) the relationship between α-amanitin resistance in rat myoblasts, and myogenesis has

been examined and it has been reported that when RNA polymerase II has mutated in such a fashion as to promote α-amanitin resistance there results pleiotropic alterations in the mutant's ability to express myogenic traits; implicating thereby RNA polymerase II as an enzyme involved in the control of myogenesis. Mutagenesis and carcinogenesis studies with some carcinogens have also been conducted and it was shown that the frequency of spontaneous and induced mutagenesis for three markers is similar in human and hamster cells. The frequency of the transformation step is much greater than the frequency of mutagenesis and primary hamster cells and much greater than the frequency of transformation in human cells. Mutagenesis in cells from Fanconi's anemia patients, on the other hand, was much lower than normal.

At John Hopkins University (NO1-CP-55713) further quantitative confirmation was found for the contractors previously expressed findings that transformation proceeds via numerous steps to malignancy. The mutation frequency in these cells was estimated for two loci and found to be 25-544 times less than the transformation frequency, but comparable to that frequency observed for actual conversion to malignancy. The distinction between the two events is the multi-step, progressive nature of neoplastic transformation. In addition, these workers have been able to induce somatic mutation and morphological transformation by treatment of cells with 5-bromodeoxyuridine followed by near ultraviolet irradiation. In these experiments it seems likely that the only cellular target was the DNA. DNA synthesized in different periods of S phase showed different sensitivities to somatic mutations and to neoplastic transformation, thereby indicating specificity of the DNA damage leading to neoplastic transformation.

At the Wistar Institute (NO1-CP-55655) the BALB/c 3T3 cells used by them gave high rates of spontaneous transformation and this cell system could not be used for quantitative comparisons of the relationship of mutagenesis to transformation. These studies indicate clearly that the nature of the cells under study contribute significantly to the outcome of mutagenesis in carcinogenesis experiments.

At the Institute for Cancer Research (NO1-CP-33367) it was found that the carcinogens benao[a]pyrene, 3-methylcholanthrene and dimethylbenzanthracene are highly effective mutagens of approximately equal potency when compared at similar extents of in vivo DNA reaction, suggesting, therefore, that their varying carcinogenic potency is related better to their different degrees of metabolism and DNA reaction rather than to any variation in the nature of DNA modification induced. Studies on the metabolic products derived from 3-methylcholanthrene confirmed that the 9,10-diol-7,8-epoxide was the ultimate carcinogen, an analogy with the findings with benzo[a]pyrene. It has further been found that the anti-BP-diol epoxide reacts in aqueous solution with DNA at the N-7 position of guanine in addition to the N-2 position reported earlier. The N-7 guanine base product is hydrolyzed from the DNA more readily, making this product harder to detect.

CONTRACT NARRATIVES

CHEMISTRY OPERATIONAL UNIT

October 1, 1977 through September 30, 1978

ALBERT EINSTEIN COLLEGE OF MEDICINE (N01-CP-55606)

Title: Studies on Microsomal Enzyme Systems Metabolizing Polycyclic Hydrocarbons in Experimental Animals and Humans

Contractor's Project Director: Dr. Jack Peisach

Project Officer (NCI): Dr. Paul Okano

Objectives: The purpose of this study is to establish a magnetic assay for the specific cytochrome(s) P-450 associated with aryl hydrocarbon hydroxylase in intact liver and in liver microsomes. This assay is based on the fact that magnetic heterogeneity indicative of the presence of a mixture of cytochrome P-450, could be spectrally resolved using low temperature EPR spetroscopy. This physical chemical approach will not only lead to the formulation of a useful clinical assay for the cytochromes in liver, but could also lead to a fundamental understanding of the structure of the protein and its relationship to the enzymatic activity. One of the purposes of the study is to elucidate how each of the cytochromes P-450 in liver contributes to the overall enzymatic activity. In addition, the relationship between substrate activation of the cytochrome, accompanied by a spin state conversion from low spin to high spin and enzyme induction with carcinogens, such as 3-methylcholanthrene, can be differentiated.

No understanding of enzyme function is complete without the determination of the structure of the active site of the protein, which entails, in part, the heme and ligand structure. Here too, an analysis of EPR data conveys this type of information.

Finally, since reduction followed by O_2 binding to cytochrome P-450 are reactions required for enzyme function, elucidation of the functional differences of the various cytochromes P-450 are made on the basis of azo reduction, a reaction that is slow enough to be followed optically so that the individual heme components of microsomal electron transport as well as the substrate itself can be studied. In this way, the relation of reduction and ligand binding properties of specific cytochrome P-450 as they relate to aryl hydrocarbon hydroxylase can be mechanistically resolved.

Major Findings: Low Spin Cytochrome P-450 - In the ferric state, low spin cytochromes P-450 exhibit EPR properties characteristic of heme ligated to a sulfur ligand with g values near 2.4, 2.2 and 1.9. No other naturally occurring low spin heme compounds have EPR spectra within this same range of g value. Recently, it has been suggested from a crystal

field analysis of EPR data that all low spin ferric cytochromes P-450, regardless of source, have a unique ligand structure, consisting of a mercaptide sulfur and an imidazole nitrogen presumably from a cysteinyl and a histidinyl residue, respectively. Although others contend that the ligand trans to sulfur is either water or an hydroxyl side chain of an amino acid, five nitrogen and single sulfur coordination has been confirmed by the K. Hodgson using X-ray absorption fine structural studies. Furthermore, nuclear modulation studies demonstrate that an imidazole is indeed bound to the heme, which differs from the imidazole of various low spin hemoproteins in that the remote nitrogen is not hydrogen bonded. A general treatment of the problem of metal-imidazole nitrogen magnetic interactions has been performed based on nuclear modulation studies. One can explain the metal nitrogen coupling on the basis of the quadrupolar interaction and an AI.S term of the spin Hamiltonian. The applicability of this approach to cytochrome P-450, all other hemoproteins and paramagnetic metalloproteins in general, is currently under investigation. On the basis of this work, both qualitative and quantitative information of imidazole linkages at metal binding sites may be obtained. For example, for a series where at least a single imidazole is demonstrated in all, one where the EPR spectrum is lacking in a visible nuclear hyperfine coupling with the Cu(II) nucleus. In these cases, a structure consisting of Cu(I)→ SR charge transfer is suggested. This is similar to the RS → Cu(II) charge transfer previously suggested for Type I copper and confirmed by the contractor with the linear electric field effect for azurins, plastocyanins and the blue copper oxidases, but here the level scheme metalligand molecular orbitals have been altered.

It is this same property of electron donating and accepting character of sulfur, modulated by the protein moiety that makes cytochromes P-450 unique in hemoproteins. It is on this basis that redox optical and magnetic properties of the various microsomal cytochromes P-450 are different from one another and explains why the EPR ascribed to cytochrome P-450 in microsomal preparations suggest heterogeneity of structure since some of the EPR features in various regions of the spectrum show more than one component. It is believed that this heterogeneity arises from protein differences, the heme ligand complex being fixed. The presence of multiple forms of cytochrome P-450 has previously been observed chromatographically, optically as well as magnetically. Immunological differences were also observed among different species. Patterns of induction and the enzyme activities also were the basis for the suggestion of multiple forms of cytochrome P-450's. Thus, it is likely that when there is a difference in drug metabolizing activities, there is an alteration in relative abundance of different cytochrome P-450 species.

Cytochrome P-450 as an Azoreductase - Cytochrome P-450 is a mixed function oxidase in that molecular oxygen is both reduced to water and is also inserted into substrates. One might consider this protein, then as an oxygen reductase. It is this reductase property that has been of interest over the last year for three reasons. The first, is that a clue to oxygen fixation will be elucidated from these studies. The second is that a sensitive assay can be developed for the cytochrome, hopefully, more

sensitive than those employing optical properties of the CO derivative of the protein per se. The third is that an assay will be developed differeniating cytochrome P-448, which is associated with aryl hydrocarbon hydroxylase from the general mixture of cytochrome P-450. The first study relevant to these problems is the demonstration that microsomal azoreductase, an enzyme activity associated with cytochrome P-450, is almost completely attributable to the cytochrome.

As a further proof that azoreductase is cytochrome P-450 and P-448, some experiments with inducible and non-inducible mice were performed. In the "inducible" C57B/6J strain of mice, 3-MC and PB pretreatment causes an increase in cytochrome P-448 and P-450 levels, respectively, which is directly proportional to the increase of azoreductase activity. However, in the "non-inducible" DBA/2J strain of mice, only PB treatment causes the increase both in cytochrome P-450 levels and azoreductase activity, while 3-MC had no effect. These experiments suggest that the P-450 type cytochromes are responsible for azoreductase activity in liver microsomes.

As part of the contract, a sensitive assay for cytochrome P-450 which depends on the bleaching of a highly colored azo dye was developed. The rate of color loss is directly related to the cytochrome content. By limiting the amount of reductant in the system, we are able to differentiate between cytochrome P-450 and P-448 on the basis of differences of off rates of CO from the CO bound hemoproteins. Thus, a simple spectrophotometric assay that does not require extraction procedures could be employed.

The cleavage of the azo linkage may be the basis for a new micro assay for cytochromes P-448 and P-450 which is based on fluorescence. In specifics, one can perform the azoreductase reaction either using a fluorescent azo compound or more likely, an azo compound that breaks down into fluorescent products. In this way, the sensitivity of the assay can in theory be improved by at least an order of magnitude.

Electron Transfer Between Cytochrome b_5 and Cytochrome P-450 - Although cytochrome b is not required in a reconstituted hydroxylating system with NADPH-cytochrome c reductase and cytochrome P-450, its implication in microsomal electron transport has been under dispute for about ten years. Using the azoreductase spectrophotometric assay, we have shown that the kinetics of cytochrome P-450 reoxidation by amaranth is followed as an independent kinetic event by the reoxidation of cytochrome b_5. Thus, cytochrome b_5 is in the electron transport chain separating microsomal flavoprotein and cytochrome P-450.

Significance to Biomedical Research and the Program of the Institute:
The significance of the contract study is that a new technique is being developed as a biological tool that extends EPR spectroscopy from applicability to the study of the first coordination sphere of the metal ion up to as far away as the third. Studies on azoreductase elucidate aspects on microsomal electron transport and can be used as a differential assay for cytochrome P-450 and cytochrome P-450 not requiring time consuming extraction procedures.

Proposed Course: It had been noted that reports in the literature had stated that riboflavin protects against chemical carcinogenicity from azo compounds. Interest was stimulated then, in the effect of flavins on azo reduction. It was found that FMN, FAD, riboflavin as well as methyl viologen, all markedly stimulated microsomal azo reduction. The mechanism for the stimulation is one in which added flavins are reduced by microsomal components, either cytochrome P-450, NADPH, cytochrome c reductase or both and the facilitation of electron transfer from the microsomes is increased.

Date Contract Initiated: August 4, 1974

Current Annual Level: 0

AMERICAN HEALTH FOUNDATION (NO1-CP-55639)

Title: Metabolism of Carcinogenic Compounds

Contractor's Project Directors: Dr. Emerich S. Fiala
Dr. Stephen S. Hecht

Project Officer (NCI): Dr. Elizabeth K. Weisburger

Objectives: The objectives of this program are to clarify the metabolism and mechanism of action of the carcinogen o-toluidine. Having identified the major in vivo metabolites of this compound in the rat, hamster, mouse and guinea pig during the first phase of this program, the problems now to be addressed include a) the identification of minor metabolites produced in vivo; b) the nature of the metabolites produced during the incubation of selected tissues with o-toluidine or its derivatives in vitro; c) the characterization of possible interactions of the metabolites with important cellular macromolecules both in vivo and in vitro. The results obtained will be significant not only in delineating the mode of action of the carciongen o-toluidine, a major industrial product, but also in understanding the peculiar biological significance of the ortho-methylamine configuration in other arylamine carcinogens.

Major Findings: In in vivo studies, the following non-conjugated urinary metabolites of o-toluidine were detected: N-acetyl-o-toluidine, o-aminobenzyl alcohol, N-acetyl-o-aminobenzyl alcohol, 2-nitrosotoluene and 2,2'-dimethylazoxybenzene. In addition, by means of Sephadex LH-20 chromatography and enzymatic hydrolysis, the following conjugates were identified; the glucuronides of 4-amino-m-cresol, of N-acetyl-4-amino-m-cresol and of anthranilic acid; the sulfate esters of 4-amino-m-cresol and of N-acetyl-4-amino-m-cresol, and the sulfamate of o-toluidine. In contrast to aniline, for which hydroxylation occurs in the ortho, meta and para positions, hydroxylation of o-toluidine in vivo appears to occur exclusively para to the $-NH_2$. Since o-toluidine is carcinogenic only at very high levels, the relationship between dose level and urinary excretion of the possible proximate carcinogen o-nitrosotoluene was studied.

A direct linear relationship between excreted 2-nitrosotoluene and administered o-toluidine exists between 25 mg/kg to 200 mg/kg. At the higher dose level, approximately 72 nmoles of the nitroso derivative is excreted in 18 hrs. With increasing dose, also, the relative amount of sulfate conjugates in the urine decreases; however, both the relative and the absolute amounts of o-toluidine sulfamate in the urine increase.

The nature of the o-methylamino effect was examined through mutagenicity studies on aniline, o-toluidine, and likely activated metabolities of these compounds. The following compounds were tested for mutagenic activity in S. typhimurium TA 100 and TA 1535 with and without activation by hepatic supernatants: aniline, N-phenylhydroxylamine, N-phenylacetohydroxamic acid, nitrosobenzene and o-toluidine, N-(2-methyl-phenyl)hydroxylamine, N-(2-methylphenyl)acetohydroxamic acid, 2-nitrosotoluene. In strain TA 100 with activation, significant mutagenic activity was observed only with N-(2-methylphenyl)-hydroxylamine and 2-nitrosotoluene, which are both metabolites of o-toluidine. None of the compounds were mutagenic in this strain without activation. In strain TA 1535, the results were similar, except that activity was also observed for N-(2-methylphenyl)-acetohydroxamic acid, without activation. These results demonstrate that the presence of the o-methyl group enhances mutagenicity as well as carcinogenicity in this series. To test the possibility that this enhancement could be due to anchimeric assistance by a metabolically formed o-hydroxymethyl group, N-(2-hydroxymethylphenyl)hydroxyl-amine was also assayed. However, this compound was not mutagenic in these strains of S. typhimurium.

Significance to Biomedical Research and the Program of the Institute: o-Toluidine, a major industrial chemical, produced annually on a tonnage basis, induces subcutaneous and urinary bladder tumors when fed to male rats and causes tumors of the spleen and liver in mice. Under identical conditions, m-toluidine is inactive and p-toluidine shows only marginal activity. The observation that o-toluidine is more active than the other two isomers or than aniline is a further example of the unique property of an orthomethyl amino group in increasing carcinogenicity. This interesting effect, which has been observed with at least four other known animal carcinogens provides a good lead to understanding the mechanism of action and organospecificity of certain aromatic amines. o-Methylaromatic amines generally are carcinogenic to the colon, breast, or urinary bladder depending on species and sex of the animal tested. Thus, metabolic studies designed to elucidate the activation and detoxification pathways of o-toluidine are relevant to mechanisms or carcinogenesis for three of the major human cancer types.

While there is now no evidence that human exposure specifically to o-toluidine had led to adverse effects, including cancer, it is pertinent to point out that historically the bladder cancers originally seen by Rehn were named "Aniline" cancers. Although it cannot be excluded that these cancers were due to higher homologs, it is possible that o-toluidine, recently discovered to be a bladder carcinogen in an animal system, may also have played a role.

Proposed Course: In view of the observed mutagenicity of N-(2-methylphenyl)-hydroxylamine and of 2-nitrosotoluene, the latter will be tested in a long-term assay for carcinogenicity in F-344 rats, and its metabolic products in vivo and in vitro, as well as binding to nucleic acids and proteins will be examined. Trapping experiments with nucleophilic substrates will be carried out to determine the nature of the electrophilic intermediates derived from metabolic transformations of o-toluidine, N-(2-methylphenyl)-hydroxylamine, and 2-nitrosotoluene.

Date Contract Initiated: June 30, 1975

Current Annual Level: $68,000

BRITISH FOOD MANUFACTURING INDUSTRIES RESEARCH ASSOCIATION (N01-CP-43337)

Title: The Development and Application of Methods for N-Nitroso Compounds and their Precursors in the Environment

Contractor's Project Director: Dr. Clifford L. Walters

Project Officer (NCI): Dr. Larry K. Keefer

Objectives: To develop a method for the determination of total N-nitroso compound concentration in solid phase samples without prior extraction.

Major Findings: The procedure under development has been further modified. As a result, it has been possible to carry out repeatability and reproducibility studies on much lower levels of a representative non-volatile nitrosamine (N-nitrososarcosine). In its modified form the method is as applicable to N-nitroso derivatives of strongly basic amines such as dimethylamine as to those of very weakly basic amides and amines (e.g., diphenylamine).

Many nitrogenous compounds other than nitrosamines and nitrosamides can potentially be formed from nitrite in a biological matrix which could well give rise to volatile products yielding a response in the chemiluminescence method under development. Procedures have been devised whereby many such compounds can be differentiated from N-nitroso compounds. During these processes, amines present in a biological matrix could be subjected to a small extent of artifactual nitrosation, but this can be prevented through the use of an inhibitor such as ascorbyl palmitate.

Significance to Biomedical Research and the Program of the Institute: An association has been noted in several countries between high levels of nitrate intake and some types of human cancer, particularly were bacterial action permits its ready reduction to nitrite, as is the case in the achlorhydric stomach or the infected bladder. Some nitrosatable amines present in biological systems yield N-nitroso derivatives which can be separated from their natural matrices on the basis of their volatility or extractability whilst other derivatives are extracted with great diffi-

culty, if at all. Most N-nitroso derivatives are carcinogenic or can lead to such carcinogens by transnitrosation; thus a simple and specific method of determination of non-volatile compounds of this type would permit the extension of epidemiological studies based on the exposure of different populations to such compounds, in accordance with the objectives of the Institute in pinpointing any relationship between environmental agents and cancer in man.

Proposed Course: Continued efforts will be made to discriminate false positives (such as pseudonitroles, thionitrites and thionitrates) potentially found in a biological matrix from authentic nitrosamines and nitrosamides. Analysis of codfish spiked with compounds will be completed, and the method will be further extended to other food matrices. Pre-formed N-nitroso compounds will be determined in biological samples of concern.

Dated Contract Initiated: June 1, 1974

Current Annual Level: $53,255

CALIFORNIA, UNIVERSITY OF (SAN DIEGO) (N01-CP-33232)

Title: Study of Pulmonary Tumors in Mice for Carcinogenic and Co-Carcinogenic Bioassay

Contractor's Project Directors: Dr. Michael B. Shimkin
Dr. Jeffrey C. Theiss

Project Officer (NCI): Dr. Elizabeth K. Weisburger

Objectives: The objectives of the project are twofold: (1) to test environmental compounds for carcinogenic activity using the strain A mouse pulmonary-tumor-induction-technique and (2) to evaluate the effect of environmental compounds on the induction of pulmonary tumors in strain A mice by known chemical carcinogens.

Major Findings: (1) Tumorigenic Activity of Substituted Organo Halides - 10 monochlorinated and 18 monobrominated derivatives of alcohols, esters, ethers, carboxylic acids, ketones and amines, containing 2 to 4 carbon atoms, were assessed for tumorigenic activity by the pulmonary-tumor-induction-technique. Four compounds, 4-chloro-1-butanol, 3-chloropropionic acid, 2-bromoethanol and 2-chloro-N,N-dimethyl ethylamine-HCl, showed significant tumorigenic activity. Ethyl chloroacetate, 3-chloropropene, 3-chlorobutyric acid, 3-bromopropionic acid and 3-bromopropylamine-HBr showed borderline tumorigenic responses.

Each of the compounds which were tumorigenic contained a nucleophilic substituent, such as a hydroxyl group, a carboxylate ion, or an amino group. In each case a conformation of the molecule may be envisioned in which the nucleophilic substituent stabilizes the electrophilic portion of the molecule. Each of the substituted compounds demonstrated tumorigenic

activity at lower doses than similar organo halides tested previously. Thus, nucleophilic substitution of organo halides appears to increase tumorigenic potency.

The high percentage (15/18) of brominated compounds which were not tumorigenic as compared to the chlorinated compounds (4/10) was surprising, since on the basis of chemical activity and previous carcinogenesis studies the brominated derivatives would be expected to be more tumorigenic. A comparison of the maximum tolerated doses of bromo and chloro derivatives of identical organo halides may explain this apparent discrepancy. Except for the 3-halo propionic acid derivatives, in which toxicity may be due to the acid moiety, the maximum tolerated dose of the bromo derivative was substantially lower than the chloro derivative. Thus, the greater toxicity of the brominated compounds may mask potential tumorigenic activity in some instances.

(2) Inhibiting Effect of Caffeine on Spontaneous and Urethan Induced Lung Tumors in Strain A Mice - The i.p. injection of caffeine (8, 20, and 40 mg/kg) thrice weekly for 8 weeks suppressed the development of spontaneous pulmonary tumors in strain A mice. The same caffeine injection scheme suppressed urethan induced lung tumor development when caffeine treatment started one week before urethan administration, but this suppression was not significant when caffeine treatment was initiated one week after urethan injection. The most pronounced suppression of lung tumor formation occured when caffeine was given as only 2 injections, 3 hours before and 3 hours after urethan. The incorporation of (^{3}H)thymidine into lung tissue DNA of caffeine treated mice was impaired at the time of urethan administration. Also, caffeine partially antagonized the effects of urethan on lung tissue, as measured by (^{3}H)thymidine incorporation studies. One interpretation of these results is that caffeine induced suppression of DNA synthesis interferes with pulmonary adenoma induction by decreasing the affinity of lung tissue DNA for urethan. The finding that chronic caffeine treatment produced continued supression of (^{3}H)thymidine incorporation into lung tissue DNA suggests that caffeine induced inhibition of spontaneous pulmonary adenoma formation is due to a general supression of lung DNA synthetic activity.

Significance to Biomedical Research and the Program of the Institute: Organo halides are widely used compounds in medicine, agriculture, and industry. The finding that nucleophilic substitution of organo halides increases tumorigenic potency will be of help when estimating the potential carcinogenic activity of organo halides on the basis of structure.

Also, the finding that the acute toxicity of the organo bromides may mask the tumorigenic activity in some instances will be a factor in the estimation of potential carcinogenicity of chemicals based on in vitro mutagenicity and carcinogenicity tests. In some instances, very active compounds in vitro may not demonstrate carcinogenicity in vivo due to the severe acute toxicity produced by these compounds.

The finding that the anticarcinogenic effect of caffeine may be due to interference with the toxic effects of urethan on mouse lung tissue may also be of importance. The presently held theory for the anticarcinogenic effect of caffeine is that it actually potentiates the acute toxicity of carcinogens due to inhibition of post replication repair. It is much more appealing to envision the anticarcinogenic effect of an environmental agent being due to supression of toxicity rather than to enhancement of the toxic effects of carcinogens.

Proposed Course: (1) Several groups are presently being tested for carcinogenic activity by pulmonary tumor bioassay. A group of sulfonic acid derivatives of naphthylamine which are used as dye-stuff intermediates is in progress. A structure-activity study of aniline mustard derivatives is under way to determine if altered structure leading to increased distribution throughout the organism leads in turn to increased carcinogenic activity. A series of antineoplastic agents is being tested to determine if the capacity to inhibit the growth of solid tumors is coupled with the carcinogenic potential of these agents toward solid tissue. A study may also be initiated of several organic contaminants of U.S. drinking waters which have been demonstrated to be mutagenic to bacteria.

(2) Studies of the anticarcinogenic effects of environmental compounds will be expanded using the same approach as that used with caffeine. In these studies a series of compounds which have been demonstrated to inhibit the spontaneous development of pulmonary adenomas in mice will be tested to determine if these compounds antagonize the effects of known chemical carcinogens.

Date Contract Initiated: June 22, 1970

Current Annual Level: $200,000

CALIFORNIA, UNIVERSITY OF (SAN FRANCISCO) (NO1-CP-43239)

Title: The Selection and Isolation of Specific, Conditional Mutant Mammalian Cells with Altered Regulation of the Purine and Pyrimidine Biosynthetic Pathways

Contractor's Project Director: Dr. David W. Martin, Jr.

Project Officer (NCI): Dr. John L. Bader

Objectives: To select, isolate, and characterize from cultured mouse lymphoma cells conditional mutants with altered regulation of purine and pyrimidine metabolism.

Major Findings: From chemically mutagenized mouse lymphoma (S49) cells in continuous culture we have isolated and characterized mutants with abnormally regulated de novo purine synthesis and others with abnormalities in their de novo pathways of pyrimidine nucleotide synthesis.

The cells of a clone termed AU-100 have 3- to 5-fold elevated intracellular levels of inosine and guanosine nucleotides and of PPriboseP, but they produce purines de novo at only 30% the normal rate. The primary defect in these cells is an 80% reduction of their level of adenylosuccinate synthetase activity. These mutant cells provide an important insight concerning the regulatory role of PPriboseP glutamyl amidotransferase in vivo and the chemical nature of its feedback inhibitor. Another isolated mutant S49 cell has two-fold elevated adenylosuccinate synthetase activity.

A clone of cells designated AU-11 has 4- to 5- fold elevated levels of activities of the two complexed pyrimidine nucleotide synthetic enzymes, orotate phosphoribosyltransferase and orotidylate decarboxylase. Other clones (e.g., FU-1-2) of cells have been isolated and characterized to possess only 40-50% of the normal level of these same two enzymes. These mutants have been used to advantage in understanding the normal regulation of pyrimidine nucleotide synthesis as well as the metabolism of the clinically efficacious cancer chemotherapeutic agent, 5-fluorouracil.

Mutants incapable of transporting aza-substituted pyrimidine nucleosides have been isolated and characterized. Along with our previously described mutants defective in the facilitated transport of ribo- and deoxyribonucleosides of purines and pyrimidines, these mutants have permitted the demonstration that aza-substituted nucleosides are transported by a cellular function(s) genetically distinct from that transporting naturally occurring nucleosides.

Clones of mutant cells lacking the purine and pyrimidine salvage enzymes (e.g., hypoxanthineguanine phosphoribosyl transferase, adenine phosphoribosyl transferase, adenosine kinase, and thymidine kinase) have been isolated in most all combinations of 2 and 3. These multiple mutants have proved to be of great utility for furthering the understanding of specific immunodeficiency diseases and providing important insights concerning new immunosuppressive agents.

Significance to Biomedical Research and the Program of the Institute: The understanding of somatic cell genetics is fundamental to understanding malignant transformation since most current theories suggest that malignant transformations involve mutation of the genome. The development and isolation of somatic cell mutants and the application of genetic and biochemical analysis to these cells provide an experimental system for understanding the mechanisms of carcinogenesis that cannot be obtained by any other means. Well-characterized mutants are essential for an understanding of cell regulatory processes, the breakdown of regulation during carcinogenesis, and for detection of environmental carcinogens as mutagens.

Proposed Course: Many other potentially interesting mutants have been isolated and are currently being characterized at the metabolic and molecular level.

Date Contract Initiated: September 25, 1977

Current Annual Level: $117,721

DOE-NCI INTERAGENCY AGREEMENT (BROOKHAVEN NATIONAL LABORATORY) (Y01-CP-50202)

Title: Repair Mechanisms in Carcinogenesis

Contractor's Project Director: Dr. R.B. Setlow

Project Officer (NCI): Dr. David G. Longfellow

Objectives: To obtain quantitative relations between damages to DNA resulting from chemical and physical agents and neoplastic transformation.

Major Findings: The contractor uses a large colony of isogenic fish Poecilia formosa, which grow as clones, to measure the quantative carcinogenic potential of chemicals and radiations. We showed earlier that thyroid cells irridated with X-rays or UV, when injected into isogenic recipients, gave rise to thyroid tumors. The contractor is now testing 8 chemicals that mimic either UV action or ionizing radiation action in their effect on DNA for their quantitative effects in inducing thyroid tumors. So far histological analysis has shown that one treatment of DMBA gives rise to thyroid tumors and that the tumors metastisize. The contractor is also testing different UV wavelengths so as to estimate the quantitative carcinogenic effectiveness of wavelengths in sunlight. By labeling the membranes of thyroid cells with ^{125}I it was shown by radioautography that such cells migrate to the thyroid and preferentially concentrate there. This finding explains the specificity of thyroid tumor formation in these animals by injection of treated thyroid cells.

Significance to Biomedical Research and the Program of the Institute: These data permit an identification of the sensitive molecules and the nature of the changes in them that lead to tumor formation. Hence one will be able to estimate quantitatively the probability of a transformation per individual chemical change in DNA and, therefore, the quantitative hazards from chemical carcinogens.

Proposed Course: (1) Complete the UV experiments using cells from liver and spleen; (2) Repeat the UV experiments on thyroid cells using different UV wavelengths so as to obtain the action spectrum for tumor induction; (3) Complete the ionizing radiation experiments using irradiated cells other than mainly thyroid in an attempt to understand the hemorrhagic response observed earlier; (4) Transplant normal and tumor tissue from one clone to another to see how well such tissues grow in nonisogenic animals; (5) Repeat the N-acetoxy-AAF experiments and complete experiments

using other chemicals such as DMBA, 4NQO and MMS that react with DNA and are carcinogenic. 4-Nitroquinoline oxide has attributes, as judged by repair, of both UV and ionizing radiation. Methyl methanesulfonate has attributes only of the ionizing radiation type; (6) Repeat the experiments above, but use cells in culture and score for transformation by loss in contact inhibition and possible neoplastic growth in vivo.

Date Contract Initiated: June 1, 1975

Current Annual Level: $91,000

UNIVERSITY OF HAWAII (NO1-CP-75915)

Title: Cycasin and Macrozamin as Potential Environmental Carcinogens

Contractor's Project Director: Dr. Hiromu Matsumoto

Project Officer (NCI): Dr. Elizabeth K. Weisburger

Objectives: 1. To develop analytical procedures for the determination of cycasin and macrozamin in meat. 2. To determine the quantities of cycasin and macrozamin in meat derived from steers which have foraged on cycad plants.

Major Findings: Trace quantities of cycasin and macrozamin can be detected by gas- and liquid-chromatography. However, when the compounds are added to meat they cannot be analyzed because of the interfering compounds present in the meat. A clean-up procedure has been developed to remove the interfering compounds. The recovery of cycasin and macrozamin from the meat samples is still low but fairly consistent. The developed analytical procedure is capable of detecting minute quantities of the compounds and improvements will be made to increase the recovery percentage.

Significance to Biomedical Research and the Program of the Institute: There is a possibility that the glycosides cycasin and macrozamin are being introduced into the human food chain via meat from cattle which have ingested cycads. The cycads have been know since 1962 to be carcinogenic. The proximate carcinogen has been identified as methylazoxymethanol, MAM, the common aglycone of these glycosides. No chemical analysis has been conducted to test for the presence or absence of the carcinogens in meat derived from cattle which have foraged on land containing cycads. Thus, there is a need to obtain information on whether or not there are cycasin and macrozamin residues in such meat, but suitable methods for their analysis in meat are not available. Development of adequate analytical methods is needed in order to obtain, as soon as possible, the information on the carcinogen residues. The developed methods can also be used, if needed, for continuous monitoring for the presence of the compounds in suspected meat.

A thorough examination of meat derived from steers which have ingested cycads for the presence or absence of carcinogenic contaminants is needed in order to determine whether or not a source of danger to human health actually exists.

Proposed Course: Chemical analytical procedures for the determination of minute amounts of the carcinogens are being developed. Known quantities of the compounds are added to meat samples which are analyzed to determine whether or not all the added compound can be recovered. The developed procedures, when high recoveries are attainable, will be used to determine the quantities of the carcinogens present in meat derived from steers which have ingested cycad plants.

Date Contract Initiated: July 1, 1977

Current Annual Level: $39,000

HEALTH RESEARCH, INC./ROSWELL PARK MEMORIAL INSTITUTE (NO1-CP-55629)

Title: Aryl Hydrocarbon Hydroxylase in Human Lymphocytes and the Relationship to Chemical Carcinogenesis

Contractor's Project Director: Dr. Beverly Paigen

Project Officer (NCI): Dr. Paul Okano

Objectives: To determine if aryl hydrocarbon hydroxylase (AHH) inducibility is genetically determined and to evaluate or confirm previous observations that persons with intermediate or high inducibility have a higher risk of lung cancer.

Major Findings: The finding last year that AHH inducibility is no different in the progency of lung cancer patients compared to a group of matched controls suggested that genetically determined intermediate or high AHH inducibility does not predispose to lung cancer. However, the finding last year only ruled out a genetic predisposition. This contractor considered the possibility that some sort of environmental agent could alter the expression of AHH inducibility making persons more susceptible to respiratory cancer. Therefore we measured AHH in a group of cancer patients who had been treated by surgery and who were considered to be free from cancer at the time of testing. These included both lung cancer patients and a group of laryngeal cancer patients since 2 laboratories have reported that laryngeal cancer patients also have a shift in AHH inducibility. A comparison of these "cured" cancer patients compared to matched controls indicated that there was no difference in AHH activity or AHH inducibility between the 2 groups. Thus it does not appear that a person's AHH activity or inducibility predisposes them to respiratory cancer.

The metabolism of 2 drugs which are metabolized by microsomal oxidases, phenacetin and antipyrine was measured. Antipyrine appeared to be correlated with a person's AHH inducibility, but phenacetin was not particularly well correlated.

Significance to Biomedical Research and the Program of the Institute: Polycyclic hydrocarbons, an important class of environmental carcinogens, are found in cigarette smoke, polluted air, and food. These carcinogens are metabolized primarily by microsomal mixed function oxygenases. If people vary in their ability to metabolize carcinogens, if this variation is genetically determined, and if the variation affects risk to lung cancer, then several pathways are opened for cancer control. High-risk individuals could be identified and advised to give up smoking, to avoid high-risk occupations, and to seek regular medical checkups.

Proposed Course: Contract ends May 11, 1978.

Date Contract Initiated: August 12, 1974.

Current Annual Level: $42,745

HEBREW UNIVERSITY (NO1-CP-43307)

Title: Studies on Mammalian Transport Systems

Contractor's Project Director: Dr. Wilfred D. Stein

Project Officer (NCI): Dr. Tsuyoshi Kakefuda

Objectives: To follow the changes in transport properties of cell membranes as cells are transformed from the normal to the malignant state.

Major Findings: The aims of this research towards obtaining reliable data on the kinetics of transfer of cell nutrients and nonmetabolised analogues of these nutrients across animal cell membranes, using both cell lines and the transformed cells derived from these has for the most part been achieved. Hebrew University has developed a theory and experimental approach to separate out the tandem processes of transport and subsequent metabolic trapping of such nutrients. Processes in a variety of "normal" and transformed cell lines have beem compared and two important conclusions have been drawn: first, that transport is not affected when growing and non-growing, and normal and malignant cells are compared . . . at least as far as the uptake of uridine is concerned. Second, it is the trapping of uridine that is enhanced when non-growing cells are stimulated to grow and that this control of the rate of trapping is lost when cells are transformed. The contractor has been successful in moving the understanding of the problem of the relation between nutrient uptake and the transformation to the malignant state, away from the hazardous regions of membrane processes to the far more easily handled area of an intracellular metabolic step, a step which should now be able to be investigated by classical

biochemical techniques. To this end, an analysis of this process has been commenced and it has been shown that the kinetic consequence of the activation of uridine uptake by serum is the greatly enhanced affinity of the uridine trapping system towards ATP. The contractor has not yet succeeded however in demonstrating this effect in cell-free extracts.

Further, it has been shown that the inhibitors of uridine uptake that have previously been used (including a fluorescent label) affect equally both transport and metabolic trapping. Thus, there is an inhibitor and label already available for future analysis of the trapping reaction and its control. Most important, inhibition of the uptake of uridine by the uridine analogue NBMI prevents the stimulation of uptake by serum and may thus interact with the control system itself.

Significance to Biomedical Research and the Program of the Institute: Hebrew University's achievement has been to develop and apply a methodology which enables one to dissect out the tandem processes of nutrient transport and subsequent metabolism. Applying this methodology to normal (growing and resting) and transformed cells, it has been shown that it is not transport which is altered in the different states of the cells, but rather it is the control of the subsequent metabolism which is relevant to oncogenesis.

Proposed Course: Future work must clearly proceed by investigating the loss of control of uridine uptake brought about by transformation to the malignant state. This is of importance in a broad sense, since such change in an uptake system may yet be a dominant feature of such transformation and there is a need to understand the mechanism of this control, so as to reduce the differential ability of malignant cells to obtain nutrients. But it is also very important to have at hand a molecular control process that is different as between normal and transformed cells, and one which occurs intracellularly rather than at the membrane. To this end there is a need to establish whether the loss of control of uridine uptake in transformed cells leave these in the state of the high affinity for ATP (as in growing cells) or of low affinity (as in quiescent cells). One would naturally assume the former to be the case, but only by direct in situ kinetic analysis can the true situation be established. In parallel it must be known in what state the cells find themselves when treated with the inhibitor NBMI, when once again their ability to control their uridine uptake is lost. Here one might assume that the cells are held in the state of low affinity for ATP, but once again this has to be directly established. With the true nature of the transformed state clarified and using as a probe inhibitors NBMI and the fluorescent label DAMG, one would be in a good position to define molecularly the change in the cell that is brought about by transformation, as far as uridine trapping is concerned. It will be of much importance to see if this finding is generalisable to other nutrients. It is natural to assume that this is the case, since it does not seem feasible that uridine alone, of the many possible nutrients, is specifically stimulated to enter the transformed cell by such a mechanism. A small point, but one of clinical significance, is to extend work on the cancer chemotherapeutic agent CAR,

Significance to Biomedical Research and the Program of the Institute: The extension of metabolism and DNA binding studies to 3MC, and comparison with earlier work on BP, has indicated general principles relating to the chemical structure of the ultimate carcinogens which allows confident predications to be made for other carcinogenic hydrocarbons. The quantitative mutation studies following in vivo binding of hydrocarbons continues to support the role of mutagenic events in the genesis of cancer. A continuation of such studies must lead to a closer understanding of the molecular biological basis of cancer.

Proposed course: The significance of the recently identified N^7-guanine product of anti-BP-diolepoxide DNA reaction will be elucidated. The nature of the mammalian cell mutations induced by the ultimate carcinogenic metabolites of hydrocarbons will be investigated as will the molecular biological consequences of such reactions.

Date Contract Initiated: June 25, 1973.

Current Annual Level: $90,000

JOHNS HOPKINS UNIVERSITY (N01-CP-55713)

Title: The Significance to Mutagenesis in Carcinogenesis

Contractor's Project Director(s): Dr. Paul O. P. Ts'o
Dr. J. Carl Barrett

Project Officer(s) (NCI): Dr. Rufus Day
Dr. Andrew C. Peacock

Objectives: (1) To develop techniques for and an understanding of the in vitro neoplastic transformation system and somatic mutation system, employing a single cell type (Syrian embryonic fibroblasts) and the same perturbation (carcinogens, mutagens, etc.). (2) To study the mutagenic and the transformation effects of benzo(a)pyrene (BP) and its metabolites, MNNG, and incorporation of 5-bromodeoxyuridine followed by near UV irradiation. (3) To investigate the relationship between neoplastic transformation and somatic mutation. (4) To investigate the involvement of genetic and epigenetic components in both the plastic transformation and somatic mutation processes.

Major Findings: (1) Characterization of the neoplastic transformation process of Syrian hamster cells with special emphasis on the progression phenomenon in transformation - The temporal acquisition of in vitro phenotypes associated with neoplasia was examined following exposure of Syrian hamster embryo cells to a chemical carcinogen. Quantitative assays measuring morphological changes, enhanced fibrinolytic activity, and anchorage independent growth were used to detect the development of transformed cells within a population of normal hamster embryo cells. Morphological transformation and enhanced fibrinolytic activity were early

so as to obtain clinically applicable kinetic data on the transport and trapping of this compound. The contractor intends to use human fibroblast strains and physiological temperature in order to derive kinetic data which may lead to improved dosage schedules for this agent.

Date Contract Initiated: June 25, 1977

Current Annual Level: $125,000

INSTITUTE OF CANCER RESEARCH (NO1-CP-33367)

Title: Nature of the Polycyclic Hydrocarbon-Nucleic Acid Compound in Hydrocarbon Carcinogenesis.

Contractor's Project Director: Dr. Peter Brookes

Project Officer (NCI): Dr. Harry V. Gelboin

Objectives: To understand the chemistry of the reaction between carcinogenic hydrocarbons and the DNA of mammalian cells which results when these compounds are metabolised by the cells. Furthermore since it now seems probable that more than one reaction product is present in the DNA, the significance of each reaction will be considered in relation to mutagenesis and transformation.

Major Findings: The earlier suggestion that the 9,10-diol-7,8-epoxide of 3MC was the ultimate carcinogen derived from this hydrocarbon has been supported by the isolation and identification of two 9,10-diol metabolites. One of these metabolites was also hydroxylated at the 1- or 2-position of 3MC.

It has been shown that 7α, 8β-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo(a) pyrene (anti-BP-diolepoxide) the ultimate carcinogen derived from BP, reacts in aqueous solution with DNA at the N^7-position of guanine in addition to the N^2-position as reported earlier. The hydrocarbon-N^7 guanine base product is hydrolysed from the DNA with a half-life of 3 hr which may explain earlier failures to detect this product.

Using the cell-mediated V79 Chinese hamster cell mutation system and tritium labelled hydrocarbons it has been shown that at similar extents of in vivo DNA reaction the carcinogens BP, 3MC and DMBA are highly effective mutagens of approximately equal potency.

The varying mutagenic effectiveness (mutation per unit dose) of hydrocarbons, like their varying carcinogenic potency, therefore relates to their different degrees of metabolism and DNA reaction, rather than to any variation in the nature of DNA modification induced.

changes observed after treatment with benzo(a)pyrene, whereas the ability to grow in agar was delayed 32-75 population doublings after carcinogen exposure. This delay was not due to selection of a small number of cells which were present early after treatment. This development of the anchorage independent growth phenotype was found to be due to the carcinogen treatment. These observations indicate that neoplastic transformation in vitro is a progressive process through qualitatively different stages. Thus, an analogy can be drawn to the progressive nature of in vivo carcinogenesis. These results justify the study of oncogenesis in cell culture as a model for neoplastic transformation in vivo. (2) Relationship between neoplastic transformation and somatic mutation - Somatic mutation and neoplastic transformation of diploid Syrian hamster embryo cells were examined concomitantly. Mutations, induced by benzo(a)pyrene and N'-methyl-N'-nitroso-N'guanidine, were quantitated at the HPRT and the Na^+/K^+ ATPase loci and compared to phenotypic transformations measured by changes in cellular morphology and colony formation in agar. Morphological transformation was observed after a time comparable to somatic mutations but at a frequency 25-540 fold higher. Transformants capable of colony formation in agar were detected at 10^{-5}-10^{-6} frequency, comparable to that of somatic mutations, but the detection time required is longer for the former (32-75 population doublings). Neoplastic transformation induced by chemical carcinogens is more complex than a single gene mutational process. Thus, comparative study does not give experimental support to predictions of the carcinogenic potential of chemicals based on a simple extrapolation of the results obtained from conventional somatic mutation assays. (3) Development of a direct perturbation to DNA for both studies of somatic mutation and neoplastic transformation - In order to distinguish the contribution of DNA to neoplastic transformation of cells in culture, a method for perturbing only DNA was used and the efficiency of this system in causing somatic mutations and neoplastic transformation was investigated. Somatic mutation and neoplastic transformation of normal diploid Syrian hamster cells in unsynchronized cultures were induced first by incorporation of BrdU followed by near UV irradiation. Cytoxicity and single-strand DNA breaks were noted. In addition, morphological transformation of the cultures, as well as somatic mutation in the $HGPRT^+$ locus ($6\text{-}TG^r$) and in the Na^+/K^+ ATPase locus (oua^r) were dose-dependent. In addition, cells in cultures treated by BrdU followed by near UV irradiation were found to grow in soft agar and to cause tumors in animals. These experiments suggest that a direct perturbation of DNA can lead to the formation of tumorigenic cells in culture. This problem was then further investigated in synchronous cultures.

In order to demonstrate the relation between neoplastic transformation, somatic mutation, and direct perturbation of DNA, synchronous cultures of early passage Syrian hamster embryonic cells were treated with 5-bromodeoxyuridine (BrdU) and then irradiated with near UV. Cells at different periods of the S phase (5 hrs.) were treated with 1 hr. pulse of BrdU followed by irradiation. The incidence of chromosomal aberrations was highest upon perturbation in the 2nd and 3rd hrs. of S phase and less in the 1st and 5th hrs. DNA damage as measured by alkaline sedimentation showed that the size decrease was about the same upon perturba-

tion in the 2nd, 3rd, 4th and 5th hrs. and was less in the 1st hr. of S phase. The mutants of HGPRT locus (6TG resistant) were induced by perturbation in the 2nd and 3rd hrs. of S phase, while the mutants of Na^{+}/K^{+} ATPase locus (ouabain resistant) were induced in a biphasic pattern, both in early and late S phases. In contrast, the highest incidence of morphological transformation was observed when perturbed in mid-S phase, particularly in the 2nd hr., while no transformation was observed in late S phase. The synchronous cultures treated with BrdU followed by irradiation and passaged continuously for ∿ 150 days became capable of producing colonies in soft agar and tumors in newborn hamsters. These results indicate that neoplastic transformation can be induced by a direct perturbation of DNA through the treatment of BrdU plus irradiation, which also induced somatic mutations in two different loci. DNA synthesized in different periods of S phase showed different sensitivities to somatic mutations and to neoplastic transformation. Specificity of DNA damage leading to neoplastic transformation is therefore implicated.

Significance to Biomedical Research and the Program of the Institute: For the first time, the relationship between somatic mutation and neoplastic transformation of mammalian systems has been critically compared and evaluated. These studies not only add to the scientific understanding of both phenomena, but are also important in a practical way, since somatic mutation has been used frequently as an assay system for cancer risk. Also, for the first time, a system of direct perturbation of DNA for induction of neoplastic transformation has been established. Such a system is important to provide understanding of the direct damage to the genome in the initiation of carcinogenesis particularly since damage to DNA has often been used as an assay system for cancer risk.

Proposed Course: 1) To establish a systematic and statistically significant relationship between the various heritable phenotype alterations of cells in culture and tumorigenicity of these cells in Syrian hamsters. Ten or twelve characteristics associated with neoplastic transformation will be carefully measured and their occurrence in transformed cell lines will be related to the degree of tumorigenicity of the cell lines. 2) To determine whether certain somatic mutations are or are not causally related to neoplastic transformation. The contractor proposes to test a series of mutational systems, including surface protein mutations, to examine whether such mutations can prevent cellular senescence in normal cells - a step towards neoplastic transformation. It is hoped that neoplastic transformation can be obtained by a series of mutations described or defined by biochemically selective conditions, so that the biochemical pathway to transformation can be determined. 3) To develop a system to perturb the all in a way leading to neoplastic transformation but not to somatic mutation.

Date Contract Initiated: June 27, 1975

Current Annual Level: $219,200

JOHNS HOPKINS UNIVERSITY (N01-CO-45610)

Title: Studies of Mammalian Cell Transport Systems

Contractor's Project Director: Dr. Albert L. Lehninger

Project Officer (NCI): Dr. Tsuyoshi Kakefuda

Objectives: To examine specific ion transport systems of the mitochondrial and plasma membranes of normal and cancer cells and to determine in what ways they differ in function and properties. Particular attention is focussed on the energy-dependent membrane transport of H^+, Ca^{2+}, and important cell fuels and metabolites between the mitochondrial and extra mitochondrial cell compartments, as they relate to the energy metabolism and acid-base balance of normal and cancer cells. It is also proposed to determine to what extent these membrane changes are induced by oncogenic agents and to what extent they may be reversed.

Major Findings: Ca^{2+} transport by tumor mitochondria. The transport of Ca^{2+} across the plasma membrane and the mitochondrial membrane is important in tumor cell biology, not only because the cytoplasmic Ca^{2+} regulates many important intracellular activities in all types of eukaryotic cells, but also because metastases of some tumors are bone-seeking, with a special affinity for Ca^{2+}. Studies here have shown that the mitochondria from Ehrlich and other ascites tumors show very high rates of inward Ca^{2+} transport and low rates of efflux; such cells show remarkably tenacious retention of Ca^{2+} and phosphate. From these and other points of evidence it appears that the Ca^{+} concentration in the cytosol of tumor cells is differently regulated than in normal cells, possibly at a lower level. It has been found that the relative rates of Ca^{2+} influx and Ca^{2+} efflux in tumor mitochondria are independently regulated, by changes in the oxidation-reduction state of mitochondrial pyridine nucleotide coenzymes. A shift to a more reduced state depresses the rate of Ca^{2+} efflux.

The suppression of ATP formation in tumor mitochondria by Ca^{2+}. Very recently a totally new aspect of the regulation of ATP synthesis in mitochondria isolated from Ehrlich and AS30D mouse tumors has been discovered. Such mitochondria, as indicated above, have a very high affinity for and tend to accumulate Ca^{2+} at the expense of respiratory energy, thus competing with oxidative synthesis of ATP. It has been found that Ca^{2+} once it has gained entry into the mitochondrial matrix has a second and independent action, apparently inhibiting ATP synthetase directly. Thus the propensity of these tumor cell mitochondria to accumulate Ca^{2+} causes a deficiency in the overall rate of ATP synthesis, which may possibly signal an increased rate of glucose utilization by the cell.

H^+ ejection by mitochondria from normal and cancer cells. In this past year the most fundamental and important discovery made in this laboratory has come from more refined and precise measurements of the number of H^+ ions ejected from mitochondria into the surrounding medium as a pair of

electrons from respiratory substrates passes through each of the three energy-conserving sites of the respiratory chain. With three newly devised methods it has been found that 4 H^+ are ejected per pair of electrons per site by the energy-transducing electron carriers in the mitochondrial membrane. Moreover, by improved procedures it has also been established that 3 H^+ are ejected per ATP hydrolyzed, and that 4 H^+ must pass into mitochondria for each ATP molecule synthesized. These newer values for the proton stoichiometry are of fundamental importance with relation to the mechanisms by which ATP is generated by mitochondria, the power plants of the cell. These values have been observed in mitochondria from normal rat liver, heart, and brain. Very recently mitochondria isolated from Ehrlich tumor cells have been tested; they also show H^+/site ratios close to 4.0 and thus possess the same general mechanism for mitochondrial energy transduction as do normal cells. However, as pointed out in the following, the energy-coupling mechanisms in tumor mitochondria are regulated differently.

Significance to Biomedical Research and the Program of the Institute: Mitochondria are the power plants of the cell; their major energy-rich product, derived from oxidation of cell nutrients, is ATP, required for all energy-dependent cell functions. The energy yielded by oxidation of nutrient molecules is converted into an energy-rich gradient of H^+ ions across the mitochondrial membrane. This gradient of acidity can be used to make ATP from ADP and phosphate and may also be used to transport i.e., pump Ca^{2+} and other ions. Regulation of the rates of ATP synthesis and ion-pumping activites is fundamental to regulation of cell metabolism and cell growth. Recent observations shed new light on the molecular controls in mitochondria that determine the direction and rate of flow of energy in normal and tumor cells. Of special importance is a set of findings indicating that some tumor cells have a special affinity for Ca^{2+}, which may be related to the well-known tendency of some human cancers to metastasize to bones.

The project is aimed toward the goal of the Carcinogenesis Program of the National Cancer Institute, namely, to determine how membrane transport processes are altered during carcinogenesis, whether spontaneous or induced by exogenous chemical or viral agents. It may also have a long-range application to chemotherapeutic problems.

Proposed Course: The contractor proposes to examine the mechanism of stoichiometry of respiration-coupled ejection of H^+ ions from normal and tumor mitochondria to identify the molecular components in the membrane that generate the energy-rich gradients of H^+ used to make ATP and pump Ca^{2+} The effect of such H^+ ejection in the acid-base balance of normal and tumor cells and on the regulation of metabolism and energy-flow shall also be determined.

It is also proposed to extend studies of Ca^{2+} transport and release by the mitochondria of tumor cells, relevant to the cell cycle. The tendency of different cancer cell lines to show a high affinity for Ca^{2+} shall be compared. In this work virus-transformed cells shall be utilized more

extensively in order to demonstrate more conclusively that the effects observed are due to the malignant change.

The contractor plans to initiate a study of other transport systems of tumor cells which are believed to promote efflux of tumor-generated compounds capable of exerting systemic effects on neighboring normal cells.

Date Contract Initiated: June 28, 1974

Current Annual Level: $155,000

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (N01-CP-43265)

Title: Toxicity and Carcinogenicity Associated with Fungal Growth on Foodstuffs

Contractor's Project Directors: Dr. Gerald N. Wogan
Dr. George H. Buchi
Dr. Arnold L. Demain

Project Officer (NCI): Dr. Elizabeth Weisburger

Objectives: To investigate the biochemical, toxic and carcinogenic effects of aflatoxins, their metabolites and analogs; to determine the metabolic fate of aflatoxins in various species; to synthesize aflatoxin metabolites; and to isolate, characterize, and investigate the toxicity and carcinogenicity of other mycotoxins produced by molds isolated from foods or food raw material.

Major Findings: Investigations have continued into mechanisms of action of aflatoxin B . Distribution of radioactive AFB covalently bound to nuclear components has been characterized. The major DNA adduct formed by AFB metabolically activated by rat liver in vitro and in vivo has been isolated and characterized as 2,3 dihydro-2-(N guanyl)-3-hydroxy AFB . A new synthesis of aflatoxin M has been accomplished, which will provide quantities of this metabolite for experimental study. Mutagenicity and antibacterial activity of various mycotoxins were assayed in Salmonella. Mollicellins C and E were mutagenic, as were rugulosin, cyclochlorotine, lumiluteoskyrin, rubroskyrin and simatoxin.

Significance to Biomedical Research and the Program of the Institute: Evidence is accumulating that chemical products arising from microbial spoilage (particularly involving mold-damage) of foods or food raw materials can appear in the human food supply and may be associated with the etiology of liver cancer in several areas of Asia and Africa. The scope of the problem in terms of numbers of compounds and/or molds that may be involved has not yet been accurately defined, but is potentially great. This project, which is related to other NCI contracts on naturally occurring carcinogens, is designed to produce relevant information on these questions.

Proposed Course: Experiments will continue along the lines suggested by the objectives outlined above. Studies on the comparative metabolism of aflatoxins may help to delineate the mechansims involved in rather wide species differences in response to these compounds. Similarly, studies on the biochemical effects, toxicity, and carcinogenicity of analogs and metabolites may provide evidence on active forms of the agent, and on sites of action. This contract will be terminated on October 31, 1978.

Date Contract Initiated: June 25, 1962.

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (N01-CP-33238)

Title: The Interactions Between Diet and Chemical Carcinogenesis

Contractor's Project Director: Dr. Adrianne E. Rogers,

Project Officer (NCI): Dr. Lionel Poirier

Objectives: To develop in rats a bioassay model for carcinogens using lipotrope deficiency which reproduces nutritional conditions and effects under which people may be exposed and to define the dietary components responsible for alteration of carcinogenesis.

Major Findings: A semisynthetic diet which provides nutrients adequate for growth of rats but is high in fat and marginally deficient in the lipotropes choline, methionine and folic acid and several amino acids: 1) enhances induction of liver tumors by 5 of 6 carcinogens tested (AFB, DENA, DBN, AAF, DDCP but not DMN); 2) enhances induction of colon tumors by DMH; 3) does not affect induction of tumors of the stomach or urinary bladder; 4) decreases induction of mammary tumors by AAF and DMBA: 5) decreases hepatic microsomal oxidases; 6) decreases clearance of DENA and DMN from blood but increases cleareance of DBN; 7) decreases hepatic microsomal production of bacterial mutagens from AFB, and AAF; in vitro; 8) increases plasma content of alpha-fetoprotein.

Different dietary factors are responsible for the alterations of carcinogenesis cited: 1) lipotrope or amino acid deficiencies are responsible for enhancement of DENA induction of hepatocarcinoma; 2) high fat is responsible for enhancement of DMH induction of colon tumors and probably for decreased induction of mammary tumors.

Significance to Biomedical Research and the Program of the Institute: Dietary effects on chemical carcinogenesis can be demonstrated in experimental animals and are strongly indicated by epidemiologic studies in people. Development of bioassay systems in animals utilizing known dietary effects will: 1) permit definition of the dietary components which may retard or enhance chemical carcinogenesis; 2) provide models in which study of metabolism of carcinogens and other mechanisms of carcino-

genesis can be made and are related to differences in effective carcinogenesis; 3) provide a sensitive system for testing compounds which give equivocal results in normal rats.

Proposed Course: 1) Further definition of the specific dietary components responsible for the enhancement of carcinogenesis by the lipotrope-deficient diet.

Date Contract Initiated: February 1, 1972

Current Annual Level: $166,560

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (NO1-CP-33315)

Title: Environmental Occurrence of N-Nitroso Compounds

Contractors Project Directors: Dr. Steven R. Tannenbaum
Dr. Michael C. Archer

Project Officer (NCI): Dr. Elizabeth Weisburger
Dr. Larry Keefer

Objectives: The objectives of this contract are: (1) to investigate how nitrate is metabolized in man, particularly how it is converted to nitrite; (2) to investigate formation of N-nitroso compounds by deliberate nitrosation of food material under simulated gastric conditions; (3) to investigate formation of N-nitroso compounds from primary amines in saliva and gastric contents; (4) to apply the findings of objectives 1-3 to the etiology of gastric cancer in Southern Colombia.

Major Findings: Several Theromal Energy Analyzer (TEA) positive compounds are formed when grains and legumes from the high-risk gastric area of Colombia are treated with nitrite. At least some of these compounds are not N-nitroso compounds.

Treatment of primary amines with nitrite leads to the formation of a variety of products including nitrosamines. Thus, methylamine yields dimethylnitrosamine. If thiocyanate is also added to the sytem, the products formed are more complex, and may include the nitrosocyanamide.

Analytical methodology has been developed for the determination of nitrosamino acids in foods. The nitrosoproline content of uncooked bacon has been compared to the nitrosopyrolidine content of the same cooked bacon. The results suggest that preformed nitrosoproline is not the major precursor of the carcinogen.

The presence of microorganisms leads to accelerated rates of nitrosamine formation for certain amines. This effect has been demonstrated for nitrosamine formation in human saliva. However, the rate of intragastric nitrosation is still much greater than the rate of nitrosation in saliva.

A series of alcoholic beverages from Africa has been analyzed by GC-TEA for volatile N-nitroso compounds, and no samples have been found which contain greater than 50μg/liter equivalent of NDMA.

A series of gastric juice samples from the high-risk gastric cancer region of Colombia has been analyzed for pH, nitrate, nitrite, thiocyanate, chloride and several metal cations. Gastric samples high in pH are also high in nitrite when nitrate is available. The effect of pH therefore appears to be related to bacterial growth or survival in the stomach. Several cases of extremely high gastric nitrites have been observed.

Studies on nitrate metabolism in man have been extended to individuals on defined diets, including those very low in nitrate. Urinary excretion of nitrate has been found to greatly exceed nitrate intake, suggesting an endogenous supply. Analysis of fecal and ileostomy samples disclosed the presence of nitrite in the ileum, and of nitrate and nitrite in feces. Thus, it appears that nitrite and nitrate may be formed in the intestine. A suggested mechanism is the heterotrophic nitrification of ammonia. These results greatly increase the estimates of exposure of man to nitrate and nitrite, and extend the role of nitrosation to intestinal cancer.

Significance to Biomedical Research and the Program of the Institute: N-nitroso compounds may be the cause of certain types of human cancer. If conditions could be found in the environment which allow correlation of N-nitroso compound formation with a specific form of cancer, the knowledge could be applied to prevention of the disease. This project will determine the total extent to which N-nitroso compounds may be present in various environments including a cancer-rich region of Colombia. Conditions which enhance or retard formation of N-nitroso compounds in foods, beverages, human saliva and the stomach will be investigated.

Proposed Course: A major emphasis in this program will continue to be N-nitroso compound formation and occurrence in a cancer-rich region of Colombia. Research will continue on conversion of nitrate to nitrite in man, nitrosation of food materials under gastric conditions, and formation of N-nitroso compounds in saliva and gastric contents.

Date Contract Initiated: June 11, 1970

Current Annual Level: $123,200

MISSISSIPPI, UNIVERSITY OF (NO1-CP-43347)

Title: Development and Application of Methods for N-Nitroso Compounds and Their Precursors in the Environment

Contractor's Project Director: Dr. John K. Baker

Project Officer (NCI): Dr. Larry Keefer

Objectives: (1) Devėlop analytical methods for N-nitroso amino acids, (2) develop analytical methods for other N-nitroso compounds using high pressure liquid column chromatography, and (3) assist NCI contractors at the Thermo Electron Corporation in the development of the thermal energy analyzer.

Major Findings: The operating characteristics of the thermal energy analyzer detector have been extensively investigated in its use as a detector for high pressure liquid chromatography. It was found that polar nitroso compounds having very low vapor pressures had response characteristics that were markedly different from those of the volatile nitrosamines. It was found that the optimum operation temperature for the nitroso amino acids was 350° C, while the optimum temperature for the volatile nitrosamines was higher.

Analytical methods for the nitroso amino acids in meat samples were developed. Analysis of samples submitted by the World Health Organization containing several nitroso amino acids were also analyzed as part of an international program for the comparison of different analytical methods.

Significance to Biomedical Research and the Program of the Institute: At the present time, analytical methods are available only for a very limited number of cacinogenic N-nitroso compounds in food products or the environment. It is speculated that the N-nitrosopyrrolidine and other volatile nitrosamines that have been observed in cooked meat samples may be formed during the thermal decomposition of the corresponding nitroso amino acid. In order to estimate the potential production of the carcinogenic nitrosamines in the cooked food, it would be desirable to be able to determine the nitroso amino acid content of the uncooked food product.

A second function of the project was to investigate the basic operating characteristics of the TEA-HPLC system and provide this information to other NCI contractors who have been receiving these units within the last year.

Proposed Course: The contract was completed 2-28-78.

Date Contract Initiated: May 1, 1974

Current Annual Level: $32,067

MISSOURI, UNIVERSITY OF (N01-CP-75946).

Title: Retroaldol Type Fragmentation of β-Hydroxynitrosamines Which May Be Environmental Carcinogens.

Contractor's Project Director: Dr. Richard N. Loeppky

Project Officer (NCI): Dr. Larry K. Keefer

Objectives: The objectives of this research contract are:

1. To determine what chemical conditions are necessary for fragmentation of bis(2-hydroxyethyl)nitrosamine (NDE1A) and whether fragmentation occurs under conditions simulating its inadvertent employment in metal cutting.

2. To determine whether the expected fragmentation products of NDE1A [(2-hydroxyethylmethylnitrosamine (HEMN) and dimethylnitrosamine (DMN)] are found in cutting fluids before or after use.

3. To determine whether seleted other β-hydroxynitrosamines to which man might be exposed can undergo retroaldol fragmentation under conditions frequently encountered during food-stuff analysis.

4. To determine the influence on the retroaldol fragmentation of NDE1A and related compounds of factors which go beyond the scope of traditional organic chemical investigations but which may markedly affect yields of fragmentation products in cutting fluids during a machining operation or in other real or simulated environmental mixtures of potential relevance to human carcinogenesis.

Major Findings: It has been determined that bis-(2-hydroxyethyl)-nitrosamine (NDE1A) fragments to dimethylnitrosamine (DMN) and 2-hydroxyethylmethylnitrosamine (HEMN) when treated with potassium tert. butoxide (KOtBu) at 70° in ethereal solvents. The yield of each of these fragmentation products is approximately 10% after 48 hours. Of possibly greater significance, however, is the finding that N-nitrosomorpholine (NNM), 2-hydroxyethylvinylnitrosamine (HEVN) and methylvinylnitrosamine (MVN) are products of this transformation as well. HEVN is produced rapidly in yields in excess of 30% within four hours. It is subsequently converted into other products. HEMN is converted to DMN and MVN under the same experimental conditions. It has been found that treatment of N-nitrosomorpholine (NNM) with strong base results in its conversion to DMN and HEMN as well as other nitrosamines. A nitrosamine structurally close to that derived from epinephrine, N-nitrosoephedrine, fragments to DMN (5% yield) under the conditions prescribed for the analysis of nitrosamines in food samples (FDA procedure).

Significance to Biomedical Research and the Program of the Institute: Bis(2-hydroxyethyl)nitrosamine (NDE1A) has been found to contaminate certain metal cutting and grinding fluids in quantities up to 3%. Animal studies have shown this compound to be a very weak carcinogen. Work in this laboratory has shown that NDE1A fragments to much more carcinogenic nitrosamines in strongly basic solution at relatively low temperatures (70-80° C). Metal cutting fluids are mildly basic but the temperatures of their employment are much higher. This suggests that some of the estimated 780,000 workers in this country coming into contact with these fluids may be exposed to much more volatile and carcinogenic nitrosamines.

The experiments also demonstrate that the observation of low quantities of volatile nitrosamines in food samples may be the result of an experimental artifact involving the fragmentation of a nonvolatile nitrosamine which is present in much larger quantity in the food sample.

Proposed Course: The proposed course is as stated in the objectives. Immediate attention is being given to the determination of whether the "fragment" nitrosamines are found in metal cutting and grinding fluids, or are produced during laboratory modeling of their utilization, so as to properly study the chemistry of NDElA with respect to its inadvertent employment.

Date Contract Initiated: September 30, 1977.

Current Annual Level: $30,453

NEW YORK UNIVERSITY MEDICAL CENTER (NO1-CP-33279)

Title: The Isolation, Propagation, and Storage of Mutant Vertebrate Cells with Specific Biochemical Lesions

Contractor's Project Director: Dr. Claudio Basilico

Project Officer (NCI): Dr. John Bader

Objectives: (1) Isolation and characterization of temperature sensitive (ts) mutants of somatic animal cells; and (2) use of these mutants to study mutation mechanisms in somatic animal cells, and the effect of specific biochemical lesions on the regulation of cell growth and division.

Major Findings: Over 100 ts mutants of the hamster cell line BHK 21 have been isolated. These mutants exhibit low reversion and low leakiness. Their ts growth is due to a variety of defects as shown by genetic and biochemical analysis. All mutations tested are recessive. Complementation tests have assigned the mutants tested so far to 30 complementation groups, with some groups containing several mutants. The ts 422E mutant cannot grow at 39° because of a defect in the processing of ribosomal RNA precursors, leading to a block in the production of 28S ribosomal RNA. This defect is probably due to the presence of a ts ribosomal protein. Mutants ts AF8 was the first ts G1 mutant isolated. These cells become arrested at 39° in the G1 phase of the mitotic cycle. Synchronization-shift-up experiments placed the cell cycle block of this mutant in early G1, between the blocks induced by serum starvation and that induced by isoleucine deprivation. Several other mutants which at 39° become arrested in G1 have been isolated and studied. These mutants are distinct from ts AF8, and they have been used to study the factors required for the exit from a resting state in BHK cells. An improved method for the selection of DNA^-, G1 or S mutants has been developed; this method consists of synchronizing cells in G1 at 33°, then exposing them to the non-permissive temperature in the presence of FUdR as they enter the S-phase

The use of a non-permissive temperature of 37.5° for the selection was found advantageous, as it favors the isolation of mutants which have very low leakiness, and survive poorly 39.5°. Most of the mutants isolated by this method have a rapid inhibition of DNA synthesis at the nonpermissive temperature. Mutant ts BN-2 appears to be particularly interesting as it displays inhibition of S-phase traverse at 39.5°, accompanied by premature chromosome condensation. In this and other mutants, replication of Herpes virus DNA is inhibited at the non-permissive temperature, suggesting alterations in cell processes necessary for viral DNA synthesis.

Significance to Biomedical Research and the Program of the Institute: Cancer cells have altered regulation of proliferation and differentiation. Conditional lethal ts mutations have proven to be very useful to further our understanding of somatic animal cells and their regulatory mechanisms. Such mutants, in particular those affected in macromolecular synthesis, cell cycle regulation, and possibly membrane formation, are extremely valuable in studies of carcinogenesis. They should provide an understanding of the cell regulatory mechanisms and of their breakdown during neoplastic transformation, which is basic to our understanding of cancer.

Proposed Course: Future work is aimed at the following objectives: (a) the isolation and study of additional ts mutants from the BHK hamster line, particularly cell cycle mutants which at high temperature are defective in functions required for G1 or S progression. In addition the contractor has isolated and is studying ts mutants from a cell line of rat fibroblasts, F2408; (b) the further characterization of the mutants already obtained from the genetic and biochemical point of view; (c) the use of some of the mutants for specific experiments, particularly the study of the functions required for cell cycle progression, the investigation of what determines cell transition from a growing to a resting state and the study of the chromatin alteration in ts BN-2 cells and its relationship to the interruption of S-phase DNA synthesis.

Date Contract Initiated: June 26, 1971

Current Annual Level: $161,332

ONTARIO CANCER INSTITUTE (NO1-CP-43331)

Title: Isolation, Propagation and Storage of Mutant Vertebrate Cells with Specific Biochemical Lesions

Contractor's Project Director: Dr. Louis Siminovitch

Project Officer (NCI): Dr. John P. Bader

Objectives: (1) Isolation of cell lines carrying mutations affecting key cell functions such as protein synthesis, or key cell structures such as the plasma membrane; (2) Biochemical and physiological characterization of the mutant lines; (3) Examination of the properties of hybrid cell

lines obtained by fusion of cells of one mutant line with other normal or mutant lines, and the use of chromosome and DNA transfer techniques for complementation analysis, possible mapping, and for studies of chromosome behavior and gene control; (4) Extension of the techniques, as they are developed, to a variety of cell types chosen not only on the basis of their suitability for studies of the regulation of cell multiplication and differentiation, but also for other purposes, such as the detection of environmental carcinogens and mutagens.

Major Findings: Work has continued on the selection and characterization of mutants of Chinese hamster ovary (CHO) and other cells, but has also evolved into development of methods for gene transfer in such cells.

In respect to mutant selection, the contractor has now examined three distinct mutations in the protein synthesis machinery resistance, to emetine (emt^r), trichodermin (trc^r), and diphtheria toxin (dip^r). These mutations involve lesions in the 40S ribosome, 60S ribosome and EF-2 elongation factor, respectively. The latter system may be particularly useful for studies of mutagenesis in human cells since dip^r cells can be selected easily and reproducibly in such a system and the frequency is highly sensitive to mutagenesis. The contractor has also developed methods for the selection and characterization of toyocamycin (adenosine kinase) mutants in CHO cells.

Several types of studies have been undertaken to examine the question of functional hemizygosity in CHO cells. To this end it has been shown that mutations for the recessive marker, emt^r, occur much more frequently in CHO as compared to other Chinese hamster cell lines. In addition, segregation involving the wild-type allele(s) from CHO emt^r x CHO emt^s hybrids also occurs much more frequently than similar segregation from CHO emt^r and other Chinese hamster cell line hybrids. These results indicate that the emt^r locus is present in only one functional copy in the CHO cells, and similar results have been obtained for toyocamycin resistance.

Measurements of the RNA polymerase II content of α-amanitin resistant lines of CHO X other Chinese hamster lines, has shown that this locus is also probably present in only one copy in CHO cells, and in two copies in other lines.

Extremely high levels of amino acid substitution have been observed in proteins synthesized by mammalian cells under conditions of extreme amino acid starvation produced by expression of certain aminoacyl-RNA synthetase mutations or by the use of histidinol. This phenomenon will be studied in relation to aging and to malignant transformation. Mutants in the protein degradative machinery will be sought. Detailed molecular characterization of asparagine synthetase mutants will be carried out.

The relationship between α-amanitin resistance (Ama^R) in rat myoblasts and myogenesis has been examined. A number of Ama^R mutants exhibit both a delayed time of onset and a reduction in myogenesis. In addition, an increase in the ratio of the mutant to the wild-type form of RNA polymerase II in the cell brought about by growth in α-amanitin, appears

to correlate with a decreased ability of the myoblast to differentiate. These results are consistent with the hypothesis that some mutations of of RNA polymerase II to α-amanitin resistance result in pleiotropic alterations in the mutant's ability to express myogenic traits, thereby implicating RNA polymerase II as an enzyme involved in the control of myogenesis.

In previous studies it was shown that colchicine-resistant mutants (CH^R) could be isolated which were altered in their permeability and were cross-resistant to a number of other drugs. These mutants did not show any alteration in their microtubules. Recently mutants resistant to the colchicine analog, colcemid (CM^R) have been isolated, which display properties consistent with altered microtubules as their mechanism of resistance. Selections were carried out in the presence of non-ionic detergent Tween 80 (100 μg/ml) and with colcemid because it was desirable to avoid the membrane-altered mutants of the CH^R class. Stably resistant lines (2- to 3-fold more resistant than its immediate parent) could be isolated in multiple single-step selections with sequences of $2x10^{-7}$ to $1x10^{-6}$ and the mutagen ethylmethanesulfonate increased these frequencies by 10- to 50-fold. The CM^R lines are different from the CH^R lines previously isolated in at least two respects. First, after three selection steps, the relative resistance to colcemid or to colchicine was less than 10-fold, while in the CH^R lines, resistance to colchicine was greater than 100-fold. Secondly, the CM^R lines display only limited cross-resistance, and only to antitubulin drugs such as colchicine and sometimes to vinblastine, in contrast to the wide ranging pleiotropic cross-resistant phenotype of the CH^R lines. The contractor is currently investigating this question in greater detail since unique cross-resistance patterns displayed by isolates may allow classifying different CM^R mutants for further characterization.

Methods for chromosome and DNA transfer in CHO and other cell lines have been developed. Using chromosome transfer, it has been shown that: 1) the dominant markers, methotrexate (mtx^R) and ouabain (Oua^R) resistance, can be transferred with efficiencies of about 10^{-5}-10^{-6}; 2) the resulting transferents are unstable; 3) the mtx^R marker can be localized to the middle-sized class of chromosomes; and 4) integration of the marker does not occur at a specific locus. The Mtx^R locus which affects the enzyme dehydrofolate reductase has been shown to be linked to genetic loci involving two further enzyme steps in the same folate metabolic pathway. Using chromosomes from CHO cells, the contractor has delineated two steps in carcinogenesis of hamster cells in vitro. In the first step, normal cells are converted into morphologically transformed cells which are not able to form colonies in agar (aga^-) and cannot produce cancers in animals. In the second step, the above colonies can be converted into aga^+ cells by a second chromosome transfer. The latter cells produce tumors in animals.

Similar studies have been conducted with the DNA from CHO cells. DNA treated with restriction enzymes and then fractionated on RPC-5 columns can transfer the markers in the folate pathway to appropriate recipient cells. Similarly treated DNA from CHO and human cancer cells can affect the first and second steps in carcinogenesis, described earlier.

Mutagenesis and carcinogenesis studies with benzo(a)pyrene and urethane have shown that the frequencies of spontaneous and induced mutagenesis for three markers are similar in human and hamster cells. The frequencies of transformation (first step) are much greater than the frequencies of mutagenesis in primary hamster cells, and much greater than the frequencies of transformation of human cells.

The frequencies of mutagenesis in cells from Fanconi's Anemia patients are much lower than normal.

Significance to Biomedical Research and the Program of the Institute: Cancer may be regarded as a disease in which cells are genetically altered such that their regulation of cellular multiplication and differentiation is defective. Temperature-sensitive mutations affecting key functions involved in cell growth and, in particular, DNA replication and mitosis are of specific interest in respect to obtaining an understanding of the breakdown of regulation during carcinogenesis. Suitably characterized cell lines are useful not only for studies of cellular regulatory processes, but for other purposes as well. One example is the use of cell-culture systems for the detection of environmental carcinogens or mutagens. The contractor's extensive list of markers will be of considerable use in this respect. In addition, studies of the genetic basis of drug resistance are of special interest in respect to chemotherapy. For example, the finding that mutation to resistance of colchicine and to Con A engenders collateral changes in sensitivity to other drugs must be considered in contemplation of multiple drug trials, and the findings on methotrexate resistance are of particular interest because of the extensive use of this drug in chemotherapy. The several dominant markers isolated in the laboratory have already been of considerable use in studies on gene transfer which, in turn, has allowed analysis of the genetic basis of the neoplastic phenotype.

Proposed Course: To continue with the studies as outlined above.

Date Contract Initiated: January 1, 1972.

Current Annual Level: $51,891

PACIFIC NORTHWEST RESEARCH FOUNDATION (NO1-CP-55719)

Title: Metabolism of carcinogenic compounds

Contractor's Project Director: Dr. John D. Scribner

Project Officer (NCI): Dr. Elizabeth K. Weisburger

Objectives: To separate and identify the urinary metabolites of Michler's ketone and methane base, and to determine the level and mode of binding of each to nucleic acid in a target organ (liver).

Major Findings: An isolation procedure has been developed which separates metabolites of Michler's ketone from other urinary lipids, accompanied by a chromatographic procedure which can resolve at least 13 different urinary metabolites, free of background compounds. Thus, it may yet be possible to identify metabolites without the use of radioactivity. Using minimal amounts of Michler's ketone and 2-acetamidofluorene (as positive control), e.g., 28 micrograms of Michler's ketone and 9 micrograms AAF/100 g body weight, it has been found that the proportion of Michler's ketone bound to DNA is the same as the amount of bound AAF. In further AAF studies, the fraction of AAF bound to DNA decreases as the dose is increased. Comparable studies with Michler's ketone are underway.

Significance to Biomedical Research and the Program of the Institute: Michler's ketone is an aromatic amine used as a dye intermediate, and which has been found to induce liver tumors in male rats. Determination of its course of metabolism should aid in establishing a conceptual base from which to reliably predict the potential carcinogenic risk of other agents now in, or to be introduced into, the human environment. The dose-response studies on nucleic acid binding refute the concept that toxic effects of carcinogens can be reduced to zero at sufficiently low doses, and suggest instead that binding to nucleic acid does not disappear at some threshold dose of carcinogen, as postulated by various authors.

Proposed Course: The metabolite isolation studies will be continued, and extended to methane base after the Michler's ketone technique has been made fully routine. Isolated metabolites will be subjected to structural studies. Nucleic acid bearing radioactive compound will be degraded enzymatically and the digest chromatographed to further characterize the nature of binding. Future studies will also compare the distribution of binding to histones and non-histone chromosomal proteins with that of AAF.

Date Contract Initiated: July 1, 1975

Current Annual Level: $38,260

ROCHESTER, UNIVERSITY OF (N01-CP-45611)

Title: Mammaliam Cell Transport Systems

Contractor's Project Director: Dr. J. Donald Hare

Project Officer (NCI): Dr. Tsuyoshi Kakefuda

Objectives: 1) To develop methods with which to identify and isolate the proteins associated with the transport of nucleosides and amino acids in mammalian cells, principally through the use of photosensitive chemicals designed to bind covalently and selectively to putative transport proteins.

(2) To make use of both photosensitive chemicals and membrane fractionation procedures to identify, isolate and reconstruct transport systems from normal and neoplastic cells.

Major Findings: Work related to these objectives has continued in several areas progressing in parallel to each other. These are: (1) the synthesis and evaluation of the basic photochemistry of compounds to be used as photoprobes in transport systems; (2) a study of these compounds as labelled photoprobes in a defined transporting system derived from the human erythrocyte; (3) a study of the phospholipid structures of the membranes of normal and neoplastic cells and; 4) a study of amino acid transport in normal and neoplastic cells.

A variety of nucleotide, amino acid and benzamidine derivatives have been synthesized and their basic photochemistry and ability to inhibit the activity of simple macromolecules, such as enzymes, determined. In parallel to these studies several compounds including 8-azido-adenosine and N6 (azido-nitrophenyl) adenosine (ANPA) have been evaluated as inhibitors of nucleoside transport in erythrocyte ghosts. The distribution of labelled compounds such as ANPA covalently bound to proteins of the erythrocyte membrane are being analyzed currently. Techniques to remove noncovalently bound labelled photo-products of hydrophobic probes have been developed in order to reduce nonspecific radioactivity in SDS-PAGE of membrane proteins. At this point efforts of associate labelling of a specific protein with the ability of a given probe to inhibit the function of the nucleotide transport system have not been successful due in part to high background.

Recent studies of the phospholipid structure of normal and neoplastic cells in culture have shown that the elevated level of a newly described component of mammalian cell membranes, phosphotidyl threonine, is restricted to hamster cells which are either transformed by polyoma virus or are a continuous cell line with altered growth properties such as the BHK 21 line. Normal hamster embryo cells, whole hamster embryos, and adult hamster organs do not show the reciprocal depression of phosphotidyl serine and elevation of phosphatidyl threonine. Mouse embryo, 3T3, SV-3T3 and chick embryo cells, either normal or transformed by RSV, fail to show the elevation of phosphotidyl threonine, suggesting that this is a species specific response of the hamster cell to altered growth control. This is further supported by the observation that BHK 21 cells transformed by the ts-3 mutant of polyoma virus show temperature related changes in the level of phosphotidyl threonine. Further studies are in progress to define the step(s) in the pathway of phospholipid synthesis which results in the appearance of this newly recognized component.

Finally, using a radioactive surface label to define in quantitative terms the surface area of normal and neoplastic cells, studies to define the kinetic parameters of amino acid transport in normal (3T3) and neoplastic (SV-3T3) mouse cells are in progress.

Significance to Biomedical Research and the Program of the Institute: The detailed molecular mechanisms by which mammalian cells acquire nutrients for growth are not yet known. Furthermore, changes in these processes have been found to occur in cancer cells. Studies to determine the mechanism of nutrient transport in normal cells and possible changes in cancer cells could provide insight into new approaches to cancer chemotherapy as well as how cancer cells develop a selective advantage in the intact animal.

Proposed Course: This is the final year of this contract and work will stop as of July 26, 1978. An attempt will be made to complete as many studies as possible in the remaining time period, but due to the complexity of the research problems addressed by the research group, there will not be a final answer on any of the programs initiated under this contract.

Date Contract Initiated: June 26, 1974

Current Annual Level: $67,500

SOUTHERN RESEARCH INSTITUTE (NO1-CP-55721)

Title: Metabolism of Carcinogenic Compounds

Contractor's Project Director: Dr. Donald L. Hill

Project Officer (NCI): Dr. Elizabeth Weisburger

Objectives: To synthesize or otherwise acquire six designated carcinogens labeled with carbon-14 and to study the metabolic pathways of these compounds in rats in attempts to determine their mechanisms of action.

Major Findings: Synthesis of [methyl-^{14}C]4-chloro-2-methylaniline, the last of the six designated compounds, has been achieved. At 1 and 3 hours after an intraperitoneal dose of this compound, rats have relatively large amounts of radioactivity in the intestine, liver, and kidneys. In rat liver, considerable radioactivity is associated with protein, DNA, and RNA. A microsomal metabolite of 4-chloro-2-methylaniline is 4,4'-dichloro-2,2'-dimethylazobenzene. The formation of this compound very likely involves 5-chloro-2-hydroxylaminotoluene as an intermediate.

Bromoacetaldehyde has been identified as a microsomal metabolite of [1,2-^{14}C]1,2-dibromoethane following derivatization with 2,4-dinitrophenylhydrozone and isolation of the derivative on thin-layer chromatography. In in vitro tests, 1,2-dibromoethane does not bind to synthetic polynucleotides. Following microsomal activation, however, binding occurs in the following order: poly C > poly A > poly G > poly U.

Microsomal reactions utilizing 2,4,6-trimethylaniline, Michler's ketone, and reduced Michler's ketone have been demonstrated, but the products have not been identified. Radioactivity of each of these labeled substrates becomes irreversibly bound to microsomes when incubated in the presence of NADPH.

Significance to Biomedical Research and the Program of the Institute: Elucidation of the enzymatic processes of activation and mechanisms of action of carcinogens present in the human environment will allow steps to be taken to inhibit formation of active metabolites, to induce detoxifying enzymes, or to provide receptor substances that prevent binding of the active species to cellular macromolecules. Such inhibitors and inducers would be candidate anticarcinogens.

Proposed Course: To continue investigations on (1) the nature of urinary and microsomal metabolites of the six designated carcinogens, (2) the enzymes involved in carcinogen metabolism and activation, and (3) the mechanism of binding of activated metabolites to macromolecules.

Date Contract Initiated: June 30, 1975

Current Annual Level: $125,000

TEXAS, UNIVERSITY OF (NO1-CP-55604)

Title: Studies of Microsomal Enzyme Systems Metabolizing Polycyclic Hydrocarbons in Experimental Animals and Humans

Contractor's Project Director: Dr. Charles R. Shaw

Project Officer (NCI): Dr. Paul Okano

Objectives: To further evaluate and improve the short-term human lymphocyte culture system for measuring aryl hydrocarbon hydroxylase (AHH) activity in man, by studying the causes for variation which affect cell growth and enzyme activity both in normal persons and cancer patients, to determine whether such variation is intrinsically or extrinsically determined, to study the seasonal variation in AHH activity reported by the group at Roswell Park Memorial Institute.

Major Findings: Initial studies of human populations in this laboratory were directed toward comparing normal persons and persons with lung cancer, and indicated an association between higher AHH inducibilities in bronchogenic carcinoma (Kellermann, et al.). Correlation between AHH and lung cancer has been confirmed by Guirgis, et al. (1975), Emery, et al. (1978), and Rasco, et al. (1978), and extended to include oropharyngeal by Rasco, et al. (1978) and laryngeal carcinoma by Kellermann, et al. (personal communication) and Trell, et al. (1976). Paigen, et al. (1977) and Jett, et al. (1978) failed to find correlation between

AHH and lung cancer. Guirgis used a different AHH assay procedure involving radioactively labeled benzpyrene as substrate and measuring water soluble extract from the reaction products. They observed higher AHH activities in cultured lymphocytes from lung cancer patients as compared to AHH in sex matched normal controls. Recent work by this contractor has made use of both the original assay method as described by Nebert and Gelboin, and the method of Guirgis.

There remain a number of problems, possibly technical, with the lymphocyte AHH system. For example, different lots of fetal calf serum and mitogen evoked different responses in lymphocytes from different individuals. Furthermore, individuals may have subpopulations of lymphocytes which respond differently to mitogen activation and which vary in relative number from person to person and from time to time within a single individual.

Another problem, reported by the group at Roswell Park, was that there was seasonal variation, with much lower AHH activity, both inducibility and induced activity, during the winter season.

Most of the above problems have been resolved. First, by using the same lots of materials for a number of subjects, it has been possible to obtain relatively reproducible results and to get significant data on patients, as described below. Further, there has been no evidence of seasonal variation in the Houston area and this contractor is thus unable to reproduce this type of variation reported in the Buffalo area. Furthermore, collaborative studies involving examination of Buffalo material shipped to Houston and examination of Houston material shipped to Buffalo and processed by a Houston technician visiting in Buffalo has shown no evidence of such seasonal variation.

Results on over 600 cancer patients and 500 normal donors show significant differences between the two groups. Patients with bronchogenic carcinoma and oropharyngeal squamous cell carcinoma both had significantly higher AHH inducibilities than normal donors. Patients with other types of cancer (colon, melanoma, leukemia, breast and uterus) had AHH inducibilities indistinguishable from the normal population.

Of considerable interest is the question of the relationship between AHH measured in cultured lymphocytes and AHH activity in the lung itself. The contractor previously reported inducible activity of AHH and pulmonary alveolar macrophages (PAMs) obtained by pulmonary lavage. Recently, in collaborative studies with the contractor, McLemore, et al. have extended the PAM studies to include studies of surgically excised lung tissue obtained from cigarette smokers with and without lung cancer. While AHH activity in PAMs and cultured lymphocytes showed a good correlation, there was no correlation between these values and the AHH in normal lung tissue obtained from seven patients with lung cancer. On the other hand, in seven control (non-cancer) subjects, there was good correlation between lung AHH and lymphocyte and PAM AHH. The explanation for these differences is not known.

Significance to Biomedical Research and the Program of the Institute: The work offers the possibility of contributing to the understanding of the biochemical and genetic basis for susceptability to certain chemical carcinogens. It also offers the possibility of predicting what persons are susceptible to such carcinogens. Further, it may provide direction for intervention in the development of chemically induced cancers, by suggesting ways of intervening in the metabolic activation of these carcinogens.

Proposed Course: This contract effort ended December 31, 1977

Date Contract Initiated: November 1, 1975

Current Annual Level: $78,000

VANDERBILT UNIVERSITY (NO1-CP-65730)

Title: Non-Histone DNA Binding Proteins

Contractor's Project Director: Dr. Lubomir S. Hnilica

Project Officer (NCI): Dr. David G. Longfellow

Objectives: To develop selective large scale methods for isolation of NP proteins from rat liver and hepatomas, subfractionate the DNA-binding NP proteins into individual protein species, determine their primary sequences in order to permit the construction of interaction models with DNA and to characterize the DNA binding sites of tissue specific NP proteins.

To localize the tumor-specific NP-DNA complexes by fluorescent antibody and horseradish peroxidase bridge techniques in order to detect malignant and premalignant cells in tissue sections.

To investigate correlations between the tumor-specific DNA-binding proteins and the biological action sites of chemical carcinogens.

Major Findings: Preparative electrophoresis of the immunologically active proteins eluting from hydroxylapatite with 50 mM potassium phosphate revealed that the antigenic proteins are represented by two major and one minor polypeptide band migrating in the molecular weight range between 50-60,000. In order to obtain quantities sufficient for final characterization of the antigenic DNA binding protein, the 50 mM potassium phosphate fraction was subjected to gel filtration on Biogel P200. A fraction virtually identical to the electrophoretic 50-60,000 m.w. eluate was obtained by this procedure. This fraction contained essentially all the immunological activity. Rechromatography of this fraction of Biogel P100 demonstrated the feasibility of separating all the proteins contained in this 50-60,000 m.w. fraction.

Experiments on histoimmunological localization of the Novikoff hepatoma DNA-binding protein antigens revealed that best results can be obtained with purified IgG fraction of the antiserum. By both the immunofluorescence and horseradish peroxidase methods the antisera to normal rat liver NP-DNA complexes was localized clearly in the nucleus. Nuclear localization was also obtained with Novikoff hepatoma cells and antiserum to Novikoff hepatoma NP-DNA complexes. The Novikoff hepatoma antiserum did not localize in normal rat liver tissue. Because of the positive complement fixation of chromatins from regenerating rat liver 24 and 48 hours after hepatectomy, localization experiments were performed with tissue sections from these livers in the presence of Novikoff hepatoma antiserum. In accord with the complement fixation data the number of positively staining hepatocyte nuclei increased with time after hepatectomy and reached maximum in 48 hours. Localization experiments with livers of rats maintained on hepatocarcinogenic diet (3'methyl-4 dimethylaminoazobenzene) are in progress.

Antibodies were produced to chromosomal nonhistone protein-DNA complexes isolated from chromatins of HeLa cells synchronized in the G_1, S, and G_2 parts of the cell cycle. No cell cycle specific antigenic nuclear proteins could be detected by complement fixation of the antisera with respective chromatins. Additionally, no significant differences in complement fixation could be detected between chromatins of cells grown at low or high densities (i.e., log versus stationary phases.) It was concluded that the antigenic DNA-binding nuclear proteins of Novikoff hepatoma most likely reflect phenotypical changes associated with malignant transformation.

Significance to Biomedical Research and the Program of the Institute: The process of carcinogenesis frequently changes the transcriptional controls of individual genes. This leads to the expression of a new cellular phenotype, characteristic for the transformed cell. Work supported by this contract demonstrated that carcinogenesis changes the immunological and biochemical specificity of a small group of chromosomal nonhistone proteins (NP). This change was detected in all tested experimental malignancies and points to a change in the transcriptional controls of transformed cells. It is anticipated that the altered antigenic properties of transformed cells can be utilized for immunochemical detection of cancerous cells in sectioned tissues and biopsies. If the tissue specific chromosomal nonhistone proteins are a part of the cellular gene-regulatory mechanism, detailed knowledge of these macromolecules may lead to new approaches to the control and prevention of cancer in man.

More specifically, the contracted research should lead to improved histochemical diagnosis of malignant and precancerous cells, identification of protein species responsible for the activation and perpetuation of cancerous phenotype, identification of DNA segments (or genes) responsible for malignancy, and finally, the elucidation of transport mechanisms by which chemical carcinogens are incorporated into the cellular genome.

Proposed Course: Presently, sufficient quantities of the antigenic DNA-binding nuclear protein which is specific for Novikoff hepatoma are being accumulated. Once available in sufficient quantities, the plan is to determine the amino acid sequence and study its interactions with homologous DNA. The anticipation is that the DNA loci interacting with the tumor-specific NP antigen will lead to a gene cluster responsible for malignant transformation. Ultimately, the roles of NP proteins (these may be different from the tissue-specific NP antigen) in the mechanism of action of metabolically activated chemical carcinogens will be studied. In perspective, these studies will correlate the phenotypic changes of normal and malignant cells to the altered transcriptional controls resulting from the process of carcinogenesis.

Date Contract Initiated: February 20, 1976

Current Annual Level: $107,638

WEIZMANN INSTITUTE OF SCIENCE (NO1-CP-02217)

Title: Study of the Role of Enzyme Induction in Chemical Carcinogenesis

Contractor's Project Director: Dr. Leo Sachs

Project Officer (NCI): Dr. Harry Gelboin

Objectives: To clarify the role of polycylic metabolism in carcinogenesis.

Major Findings: 1) Transformation of Normal Hamster Cells in Benzo(a) Pyrene Diol-Epoxide -- The frequency of cell transformation was determined after treatment of normal hamster embryo cells with benzo(a)pyrene (BP) and 6 of its metabolites. These metabolites included the trans 4,5-,7,8- and 9,10-dihydrodiols; the 4-5-epoxide; and two stereoisomers of the non-K-region diol epoxides r-7, t-8-dihydroxy t-9, 10-oxy-7,8,9,10-tetrahydrobenzo(a)pyrene (diol-epoxide I) and r-7, t-8, dihydroxy c-9, 10-oxy-7,8,9,10-tetrahydrobenzo(a)pyrene (diol-epoxide II). The trans 7,8-dihydrodiol was more active than the other 2 dihydrodiols tested and was also more active than the parent hydrocarbon BP. Of the 3 epoxides tested, the diol-epoxide I was more active than the 4,5-epoxide and diol-epoxide II. The results suggest that diol-epoxide I is a major cell-transforming metabolite of BP. 2) Metabolism of the Carcinogenic Hydrocarbon Benzo(a)Pyrene in Human Fibroblast and Epithelial Cells -- Aryl hydrocarbon (benzo(a)pyrene) hydroxylase (AHH) activity and metabolism of benzo(a)pyrene to water-soluble products were measured in cultures of body fibroblasts and kidney epithelial cells from 23 different human embryos. The fibroblasts from the different embryos could be divided into 3 groups according to the amount of water-soluble products, but not according to the AHH activity. These 3 groups were not found by either assay in the cultures of kidney epithelial cells. In both fibroblast and epithelial cells, high metabolism to water-soluble products was not necessarily associated with high AHH activity. The results extend pre-

vious findings of 3 genetic groups for BP metabolism to water-soluble products in human fibroblast but not in epithelial cells and indicate that this grouping was not found in these cells by measuring AHH activity. 3) DNA Binding and its Relationship to Carcinogenesis by Different Polycyclic Hydrocarbons -- 5 different polycyclic hydrocarbons with different degrees of carcinogenicity in vivo were tested for their metabolism to water-soluble products and their binding to DNA, RNA and protein in normal embryonic hamster and BHK cells. All compounds were metabolized to water-soluble products in both types of cells and treatment of cells with aminophylline enhanced this metabolism. After and not before this enhancement of metabolism by aminophylline, there was a relationship between the degree of carcinogenicity and binding to DNA. There was no such relationship with binding to RNA or protein. The results, indicating a relationship between the degree of carcinogenicity and binding to DNA under appropriate conditions of metabolism, support the suggestion that DNA is the target for carcinogenesis by such carcinogens. 4) Control of Cloning of Normal Human T Lymphocytes by Transferrin, Albumin and Different Lectins -- Normal human T lymphocytes can be induced to form colonies with a high cloning efficiency by seeding the cells directly in agar with normal human plasma and the lectins concanavalin A, phytohemagglutinin, or pokeweed mitogen. The requirement for human plasma can be substituted, to different degrees with different lectins, by adding transferrin and albumin to fetal calf serum. This provides a useful system to identify specific deficiencies in the requirements for normal T lymphocyte colony formation.

Significance to Biomedical Research and the Program of the Institute: An understanding of these genetic and biochemical processes is necessary for understanding carcinogenesis in humans.

Proposed Course: To further clarify the relationship between the AHH enzyme system, the metabolism of carcinogenic hydrocarbons and malignant cell transformation.

Date Contract Initiated: May 26, 1970

Current Annual Level: $271,240

WEIZMANN INSTITUTE OF SCIENCE (N01-CP-33220)

Title: Alterations Produced by Chemical Carcinogens in Viral and Cellular Gene Expressions

Contractor's Project Director(s): Dr. H. Aviv
Dr. U. Littauer
Dr. M. Revel
Dr. E. Winocour

Project Officer (NCI): Dr. D. Hatfield

Objectives: The objectives are: (1) to investigate the molecular mechanisms by which chemical carcinogens enhance viral induced cell transformation; (2) to examine the effects of chemical carcinogens on cell-virus recombination events which occur during virus infection; (3) to study the effects of carcinogens on the expression of tumor viral genes; (4) to study the effect of carcinogens on the function of tRNA; and (5) to study the mechanisms by which tumor promoters inhibit cellular differentiation.

Major Findings: The major findings were: (1) The transformation of Chinese hamster embryo cells by SV40 was enhanced several fold when the cells were pretreated with 4-Nitroquinoline-1-oxide or with benzpyrene-diol-epoxide; (2) the infectivity of SV40 DNA to which benzpyrene-diol-epoxides were bound was shown to be significantly less effective in a productive infection; nevertheless, the replication of the infected DNA was normal; (3) viral RNA synthesis was severely inhibited in SV40 infected cells treated with dimethyl-sulfate, while viral DNA synthesis was stimulated; (4) tRNA isolated from ethionine treated animals was assayed functionally in vivo by microinjection into Xenopus oocytes and found to be remarkably impaired in its capacity to sustain protein synthesis; (5) in contrast to BrdU several chemical carcinogens failed to induce C-type virus particles; (6) tumor promoters (phorbol esters) which were found to inhibit the differentiation of Friend erythroleukemic cells in culture seem to inhibit the biosynthesis of globin mRNA rather than enhance the degradation of synthesized globin RNA molecules; and (7) two T-antigen polypeptides (17,000 and 90,000 m.w.) which are coded by mRNA molecules generated by splicing of a common precursor were identified in SV40 infected cells and only the larger one has the capacity to interact with DNA.

Significance to Biomedical Research and the Program of the Institute: During induction of cell transformation, chemical carcinogens may interact with genetic information of viral or cellular origin. Elucidation of the molecular mechanisms by which chemical carcinogens and their metabolites affect integration and expression of the tumor virus genome is important for understanding co-carcinogenesis. These studies can serve as a basis for new cancer screening techniques.

Proposed Course: The proposed course is: (1) to explore the nature, specificity and extent of the effect of chemical carcinogens on the recombination between viral DNA and the host chromosome during the lytic cycle and during cell transformation; (2) to determine the stage in viral expression which is affected by the binding of chemical carcinogens to viral DNA; (3) to study the relationship between carcinogen-affected biomethylation and stimulation of viral DNA synthesis; (4) to pursue the newly developed in vivo system of microinjection of tRNA into Xenopus oocytes which has paved the way for an extensive study of the effects

of carcinogens on tRNA; this method could easily be adapted as a screening method for various carcinogens; (5) to study the nature of the inhibitory effect of the tumor promoter on biosynthesis of globin mRNA; (6) to examine the possibility that recombination occurs between endogenous C-type genes and exogenously infected viruses using recently developed microscopic techniques of heteroduplex and R-looping; and (7) to examine the nature of the interaction of T-antigen with DNA and the mode by which these two antigens are produced.

Date Initiated: June 1, 1976

Current Annual Level: $298,000

WISCONSIN, UNIVERSITY OF (NO1-CP-65734)

Title: Induction of Aryl Hydrocarbon Hydroxylase in Cultured Human Lymphocytes

Contractor's Project Director: Dr. Gottfried Kellermann

Project Officer (NCI): Dr. Paul Okano

Objectives: (1) To evaluate and to determine the reproducibility of AHH induction in cultured human lymphocytes; 2) to relate the relative AHH activity and inducibility in cultured lymphocytes to the in vivo oxidation rates of certain model drugs which resemble in their metabolism that of benzo[a]pyrene and other carcinogens.

Major Findings: An excellent correlation has been found between the rate of benzo[a]pyrene metabolism in mitogen stimulated lymphocytes and the metabolic rate of antipyrine (r=0.95) and various other drugs in vivo. It opened the possibility of checking individual AHH activities by the use of a safe model drug. When patients with bronchogenic carcinoma were checked with the antipyrine in vivo test and compared to matched control subjects, significant differences were found. Lung cancer patients had significantly higher oxidation rates. Since antipyrine metabolism is under strict genetic control it is concluded that subjects with genetically increased oxidation rates have a much greater risk to contract lung cancer than subjects with low oxidation rates.

Significance to Biomedical Research and the Program of the Institute: It has been widely accepted that most carcinogens have to be enzymatically activated to exert their carcinogenic effects. Since people differ with respect to these activating enzymes it is of great importance to develop test systems that assess individual enzyme activities. These test systems can be used to identify subjects at highest risk for certain chemically induced cancers, e.g., lung cancer, and will help to prevent cancer in these individuals.

Proposed Course: Experiments to date have developed a test system that can potentially be used to identify high risk individuals for lung cancer. This contract activity ends May 17, 1978.

Date Contract Initiated: August 18, 1975

Current Annual Level: $101,455

THE WISTAR INSTITUTE (Philadelphia) (N01-CP-55655)

Title: Significance of Mutagenesis in Carcinogenesis

Contractor's Project Director: Dr. Leila Diamond

Project Officer(s) (NCI): Dr. Rufus Day
Dr. Andrew C. Peacock

Objectives: To investigate the possibility that chemical carcinogens act by inducing somatic mutations on the genome at specific sites concerned with the regulation of growth.

Major Findings: A number of clones and subclones of BALB/c 3T3 cells were analyzed and screened for their suitability for quantitating transformation induced by chemical carcinogens. All the clones showed a high propensity for spontaneous transformation and it was concluded that this cell system is not suitable for transformation-mutation studies. Consequently, attempts were made to develop a new "normal" cell line that would be suitable for this project. One clonal isolate derived from C57BL10 mouse embryos is now in the 22nd passage post-cloning. The cells of this clone have a flat morphology, maintain a low saturation density, do not grow in soft agar or nude mice, and can be transformed by 4-nitroquinoline-1-oxide. Susceptibility to transformation induced by other types of carcinogenic chemicals is being analyzed now.

Significance to Biomedical Research and the Program of the Institute: The eventual control of chemical carcinogenesis will be either through the identification of carcinogens and their elimination from the environment, or through understanding, and thereby controlling, their mode of action. Thus, an understanding of the relationship between carcinogenesis and mutagenesis is important for both practical and theoretical reasons. The approach to investigating this relationship was to obtain a system in which both events can be measured and compared under similar conditions.

Proposed Course: Contract was brought to an orderly phase-out on March 21, 1978.

Date Contract Initiated: June 27, 1975

Current Annual Level: $50,064

YALE UNIVERSITY (NO1-CP-55673)

Title: Selection and Propagation of Monosomic and Haploid Cell Lines

Contractor's Project Director: Dr. Frank H. Ruddle

Project Officer (NCI): Dr. David G. Longfellow

Objectives: To isolate and propagate monosomic, partially monosomic and haploid mouse cell populations. Teratomas are being used for the production of monosomic and partially monosomic cell lines because they are chromosomally stable near the diploid level, and their genetic constitution can be manipulated as required. Spermatids are being used to produce haploid cell lines. Basic genetic studies on the linkage relationships of murine gene loci advance these investigations.

Major Findings: New combinations of F_1 animals between inbred strains of mice have been discovered which provide an increased yield of teratoma cell populations with near diploid chromosome constitutions. Inbred strains C3H, DBA, and BALB have been compared with the F_1 combinations BALB x DBA F_1 and 129 x Ma F_1. In this procedure, six day old embryos of different genetic constitutions are implanted under the kidney capsules of isogenic host animals. It was found that BALB x DBA F_1 embryos yield highly pluripotent teratomas at a high frequency. Chromosome analyses indicate that these lines can be used as sources for monosomic cell populations. Sublines of existing strains of teratoma 6050 from Dr. L. Stevens at The Jackson Laboratory have been cytogenetically analyzed to assess their levels of monosomy. Substantial fractions of these cell populations are monosomic for chromosomes 4, 11, and 18. The presence of a monosomic 11 component is interesting and potentially useful, since the selectable genetic marker, thymidine kinase has been assigned to this chromosome.

In a series of cell hybrid and microcell hybrid experiments, eleven new gene loci have been mapped to mouse chromosomes. Biochemical markers amenable to somatic cell genetic studies are now available on 17 of the 21 genetically distinct chromosomes of the mouse.

Experiments are being continued to activate the proliferation of spermatids and sperm cells. Activation is being attempted by biochemical treatment of the haploid cell types, and by fusion of these cells with proliferating cells, cytoplasts, and erythrocyte ghosts. Particular attention is being given to the stabilization of spermatids in vitro following their proteolytic release from organized testis tissues. Combinations of treatments involving tissue culture feeder layers of various compositions (fibroblasts, Sertoli cells, etc.), addition of proteins and various macromolecules to culture medium, variation of the ionic composition of the medium, and the addition of various hormones alone and in varying combinations. In one series of experiments the useful finding was made that the treatment of spermatids with concanavalin A

stabilizes the cells with regard to subsequent treatments with polyethylene glycol. It is believed that this protocol will substantially increase the future success in hybridizing spermatids.

Significance to Biomedical Research and the Program of the Institute: Well characterized monosomic or haploid cell lines will greatly increase the sensitivity of assays for chemically induced transformation. Such cell lines will also be useful in isolating various somatic mutants such as drug resistant mutants, and temperature sensitive mutants.

Proposed Course: A number of teratoma cell lines will be analyzed in detail to establish their pattern of monosomy. Hybrids of mouse spermatids with human cells from which the human chromosomes are likely to be segregated will be made. Activation of spermatids by fusion with microcells will also be tried. A new series of experiments will be conducted on biochemical agents which may be expected to activate spermatids and sperm. These are sulfhydryl reducing agents, a variety of ionophores, and a series of biogenic polyamines. The introduction of egg extracts into sperm (and also spermatids) using erythrocyte ghosts as vehicles by means of membrane fusion will also receive heavy emphasis. These experiments will be conducted at Yale by A. Lalazar from A. Loyter's Laboratory, Hebrew University, Jerusalem. Professor Loyter, a leader in the field of erythrocyte ghost loading and fusion will also consult on these experiments during his residence in this laboratory during the period 1978-79.

Date Contract Initiated: January 18, 1975

Current Annual Level: $131,804

SUMMARY REPORT

INTERDISCIPLINARY PROGRAMS AND RESOURCES
OPERATIONAL UNIT

October 1, 1977 through September 30, 1978

The contracts in the Interdisciplinary Programs and Resources Unit consist of service projects as well as special research programs. They include three large interdisciplinary programs covering several areas of carcinogenesis research; animal and chemical resources; and projects relating to multi-disciplinary efforts that do not belong in other program areas.

Interdisciplinary Projects

The three interdisciplinary efforts are (1) the NCI program at the Frederick Cancer Research Center managed by Litton Bionetics, Inc. (NO1-CO-75380); (2) an interagency agreement with the Department of Energy for research carried out at the Oak Ridge National Laboratory (YO1-CP-50200); and (3) the program of the Eppley Institute for Research on Cancer at the University of Nebraska (NO1-CP-33278).

Frederick Cancer Research Center (FCRC)

The FCRC has as its primary objective the development of a unique national resource capable of initiating and evaluating a broad range of experimental approaches to effectively identify human carcinogens. Toward this end, the Center employs a coordinated interdisciplinary approach which consciously integrates basic and applied research. Research programs have been initiated which address various facets of the following general areas: identification of carcinogenic or co-carcinogenic environmental chemicals; investigation of host-chemical interactions; and attempts to elucidate mechanisms of carcinogenesis to permit the manipulation of factors which will block or reverse oncogenesis.

Among the major research findings resulting from this program are the following:

- A method for the routine isolation and growth of large numbers of normal breast epithelial cells has been developed using collagenase dissociation of human breast tissue which results in a cell mixture of enriched epithelial elements. These cultures grow and maintain normal morphology under the influence of physiological levels of specific hormones.

- In studies directed at factors which enhance gastrointestinal carcinogenesis, lithocholic acid, a major human fecal bile acid, enhanced the mutagenicity of 2-aminoanthracene and benzo(a)pyrene when a phenobarbital-induced rat liver S9 fraction was used.

- The potential importance of human intestinal microflora continues to be supported by the observation that the metabolism of environmental azo dyes by human intestinal bacteria has produced mutagenic metabolites.

- A new simpler method of synthesizing polycyclic aromatic hycrocarbons has been developed and applied to the synthesis of a variety of benz(a)anthracene derivatives.

- The development of high temperature nematic liquid crystals for gas-liquid chromatographic (GLC) separations of mixtures of polycyclic aromatic hydrocarbons and of their metabolites has been extended to the separation of bile acids and their metabolites.

- Monographs for the safe handling of 9 known or suspected chemical carcinogens were prepared.

- Most of nucleophile methylation observed during microsomal degradation of dimethylnitrosamine in vitro involves methyl groups originating from the medium or microsomes, rather than from the carcinogen. The nitrosamino function strongly accelerates solvolytic departure by the deposition of groups only at the β-carbon. The post-microsomal fraction of rat liver homogenates has nitrosamine metabolizing activity. New syntheses were devised for vinylnitrosamines, and for the corresponding epoxides.

- Tests of 5 nitrosamines in guinea pigs have shown that all are carcinogenic, but induce tumors in different organs in this host than in the rat.

- Findings are presented that support a generalization due to Jerina and Daly which suggest hydrocarbons, in general, are activated through bay region diol-epoxides.

- A diol-epoxide generated in the 1,2,3,4-ring of DMBA (not in the 8,9,10,11-ring as had been suggested by others) is involved in its binding to DNA. In microsomal systems, analogous to those used in conjunction with bacterial mutagenesis assays, a reactive K-region oxide, not a 1,2,-3,4-ring diol-epoxide, is generated. Mutagenicity in these studies is not based on the same properties as carcinogenicity. Different activation systems are required to properly use mutagenesis as an indicator of carcinogenic activity of hydrocarbons.

- Under conditions leading to 100% survival, DNA replication occurs on an extensively damaged template. Major differences in the repair response to chemicals of different carcinogenic potency have not been detected.

- Data suggest that the common factor amongst chemical carcinogens is their ability to generate an ionic intermediate whose selectivity permits it to modify some critical cellular receptor.

DOE-NCI Interagency Agreement (Oak Ridge National Laboratory)

An interdisciplinary research approach to solving problems of chemical carcinogenesis continues to be the major objective of this program. Research includes probes into respiratory carcinogenesis relationship of tumor cell membrane transport to DNA repair, in vitro carcinogenesis and metabolism, and activation of chemical carcinogens. Major contributions of this program include:

- Multistage carcinogenesis was demonstrated in tracheal epithelium utilizing an organ culture-cell culture technique.

- A simple and rapid in vitro system using keratinizing tracheal epithelial cell lines was developed for the study of the effective and toxic levels of retinoids.

- Nickel subsulfide was shown to be a weak but definite carcinogen for respiratory epithelium.

- A new model system, in which denuded tracheal transplants are repopulated with epithelial cells, has been developed to test the differentiative and oncogenic capacity of these cells.

- Incubation of benzo(a)pyrene with microsomal fractions of rat, mouse, and hamster liver and tissue culture cells produced derivatives which were not found when the carcinogen was incubated with intact cells.

- Amongst a series of 8 safrole derivatives, 1'-acetoxysafrole produced a typical UV-type repair response in normal human cells. Xeroderma pigmentosum cells (expected to be defective in repair after treatment with this compound) showed normal repair. The damage induced by 1'-acetoxysafrole is not a UV-type damage recognized by the UV-endonuclease system of human cells. Cyclohexylcarbamate induced many UV-endonuclease-sensitive sites in the DNA of human cells; no such sites were introduced in the DNA by 1'-acetoxysafrole. These data suggest we are observing with 1'-acetoxysafrole a heretofore undescribed DNA repair system.

- In human colon carcinoma cultures, there is a reduction of 50-70% of normal repair as compared to human fibroblasts.

- A major force driving Na^{+}-coupled α-amino-isobutyric acid (AIB) is the electrical potential across the membrane. A model was derived to study the interactions of these factors. It was provisionally concluded that the membrane potential is an important factor in growth regulation. The uptake and metabolism is altered in malignant transformed cells. Rapid

glucose metabolism of tumor cells can be controlled by the flavonoid compound, quercetin. It was shown that quercetin acts by intercalating in the lipid bilayer of the membrane, possibly by increasing its local rigidity.

Eppley Institute for Research on Cancer (University of Nebraska)

The principal objectives of this contract are the bioassay of suspected chemical carcinogens, definition of the biological and chemical mechanism of the carcinogenesis process, and the analysis of trace levels of environmental carcinogens.

The major findings are:

- Selective induction of pancreatic cancer in the Syrian hamster was demonstrated with N-nitrosobis(2-oxopropyl)amine. Ductular cells of the pancreas seem to be the most responsive to this carcinogen.

- Investigations of a possible bile reflux mechanism as a factor in pancreatic carcinogenesis contraindicate in this model the importance of biliary reflux in pancreatic oncogenesis.

- N-nitrosobis(2-oxopropyl)amine induced colorectal cancer in MRC rats. Tumors were concentrated in specific segments of the large bowel. This model may closely resemble the corresponding human disease.

- The hamster pancreatic carcinogen, N-nitrosobis-(2-hydroxy)-propylamine (BHP) and N-nitrosobis(oxopropyl)amine (BOP) were synthesized with ^{14}C-label. There were quantitative differences in the metabolism of BHP and BOP in the rat, a species in which these compounds are pancreatic carcinogens. This observation may elucidate the organotropic effects of these compounds.

- Phenylethylhydrazine (Nardil:Phenelzine), used clinically as an antidepressant, induced blood vessel and lung tumors in mice.

- Gyromitra esculenta (a mushroom) contains appreciable quantities of N-methyl-N-formylhydrazine which is extremely hepatotoxic in mice. Another mushroom, Agaricus bisporus produced tumors of lungs and blood vessels in mice.

- Succinic acid 2,2-dimethylhydrazide, a plant growth regulator used in the U.S.A., produced tumors of blood vessels, lungs and kidneys in mice.

- Studies demonstrated that conditions exist in mammalian systems for the breakdown of agaritine (constituent of the mushroom, Agaricus bisporus) to a compound that is likely to be carcinogenic.

- Different activation mechanisms are responsible for the carcinogenic activity of various polycyclic aromatic hydrocarbons (PAH).

- Syntheses of tritiated benzo(a)pyrenes were developed and they were employed to support one-electron oxidation as the principal mechanism of activation of this carcinogen.

- Peroxidase may be the activating enzyme in mammary gland carcinogenesis by hydrocarbons.

- Sulfate esters of the benzylic alcohols are strongly mutagenic towards strain TA 98 of Salmonella Typhimurium, in contrast to the parent alcohols and the nonbenzylic esters.

- The stronger carcinogenicity of 7-acetoxymethylbenz(a)-anthracene relative to that of hydroxymethylbenz(a)anthracene (BA-7-CH_2OH) was observed. These findings suggest that the activity of (BA-7-CH_2OH) may be related to the formation of a carcinogenic metabolite.

- The very rapid reaction in organic solvents may be the mechanism for formation of N-nitroso compounds under certain industrial situations (e.g., in agricultural chemicals) and in fried bacon.

- Studies suggest that diet is a determinant of in vivo nitrosation, through an effect on gastric pH.

Projects in In Vitro Carcinogenesis

In another group of projects funded from this program, the major objectives are the development of cell culture systems, which will be useful for the bioassay of environmental agents that may cause cancer, and for studies on the mechanism of chemical carcinogenesis. A contractor at Columbia University (N01-CP-23234) found that the tumor promoting phorbol esters induce plasminogen activator in normal cell cultures and produce other changes in cell phenotype that mimic those found in transformed cells. These agents were found also to be potent inhibitors of terminal differentiation. Cytologic markers and growth in agar have been demonstrated to be valid indicators of the malignant potential of rat liver epithelial cells transformed in vitro by chemical carcinogens.

The objectives of the continuing studies at the Salk Institute (N01-CP-75970) is to understand the changes in growth control, particularly changes in control of the cell cycle, that take place during chemical carcinogenesis. In this respect, data indicate that the rates of internalization and

degradation of the epidermal growth factor (EGF) are approximately the same in the normal and transformed cells. These observations indicate that it is the lower affinity of BP_3T_3 cells (benzo(a)pyrene-transformed) for EGF plus the increase in sensitivity of BP_3T_3 cells to EGF that results in less destruction of EGF and greater proliferation of the chemically-transformed cells.

Other Projects

Other projects not relating to any specific section, but of relevance and importance to the entire Carcinogenesis Program, are also supported by this program. At the Children's Hospital of Philadelphia (N01-CP-65803), a study to determine oncogenesis and other deleterious late effects of cancer therapy is being conducted. Eleven pediatric cancer centers in the U.S.A., Canada and Western Europe contribute data to this study. To date, a total of 150 new tumors in children with a first neoplasm was reported.

Non-oncogenic effects following irradiation therapy include: pulmonary restrictive disease and intestinal obstruction. In 21 children with ALL, all of whom received cranial irradiation, 9 of 16 reexamined one year later, showed consistent cognitive deficits associated with short-term memory and visual-motor perceptions.

Support Projects

The objectives of Information Planning Associates, Inc. (N01-CP-75896) are to provide management and technical support services to the Collaborative Carcinogenesis Research Program. The major contributions resulting from this contract include:

- The Carcinogenesis Research Inventory covering U.S.A. funded active projects for FY 77 was compiled.
- A compendium of scientists with their name, address and area of expertise was prepared.
- The Annual Report of the Carcinogenesis Program (July 1976 - September 1977), containing information related to organization of the program, Program Summary Reports, and Contract Narratives was prepared under the direction of NCI staff.

Franklin Institute Research Laboratories (N01-CP-65812) is providing information support services to the Human Tissue Study and Retinoid Programs.

Chemical Resource Activities

The Chemical Repository is located at the IIT Research Institute (N01-CP-55646) in a facility built to ensure safe handling of carcinogenic materials. There are now over 220 compounds available for distribution to NCI contractors and other federal agencies, with over two thousand samples having been distributed to researchers in the last year. Under Basic Ordering Agreements (BOA's), IIT Research Institute (N01-CP-65745), Midwest Research

Institute (N01 CP 65739), Southern Research Institute (N01 CP 65740), and Stanford Research Institute (N01 CP 65741) are synthesizing compounds for the Repository in laboratories designed for safe handling of carcinogens.

The synthesis of N-nitroso compounds has continued at the University of New Hampshire (N01 CP 55675). Nitrosamines react with carbon dioxide to give N-nitroso-α-aminoacids, which upon treatment with lead tetraacetate yield α-acetoxynitrosamines. Electrolytic oxidation of nitrosamines also produces these compounds. N-Nitrosocarbazole nitrosates secondary amines by a photochemical mechanism. Finally, achiral N-nitrosamines have been shown to give induced circular dichroism on interaction with chiral alcohols. The analytical chemistry services of the Midwest Research Institute (N01 CP 23270) were utilized for the assay of 3-methylcholanthrene metabolic products contained in liver and pancreas extracts by high pressure liquid chromatography, mass spectrometry and other analytical methods. To date, over 130 derivatives of benzo(a)pyrene, benz(a)anthracene, 7,12-dimethylbenz(a)-anthracene, dibenz(a,h)anthracene, and 3-methylcholanthrene have been prepared at Midwest Research Institute (N01 CP 33387).

The University of Chicago (N01 CP 33385) is continuing with the synthesis of polycyclic hydrocarbon derivatives. Molecular orbital theoretical calculations based on the perturbational method of Dewar were found to provide good correlation between predicted and observed structures of products formed during isomerization, hydration, and nucleophilic addition to arene oxides and dehydration of arene dihydrodiol metabolites of polycyclic aromatic hydrocarbons. A method for the efficient resolution of the racemic dihydrodiol metabolites of benzo(a)pyrene by means of HPLC separation of the (-)-dimethoxyacetate derivatives was devised. Synthesis of the diolepoxide derivatives of chrysene, benzo(e)pyrene, dibenz(a,c)anthracene, 7-methylbenz(a)anthracene, 7,12-dimethylbenz(a)anthracene, and triphenylene has been accomplished. The major in vivo DNA adduct formed from human and bovine explants on exposure to ^{3}H-BP was shown to arise from trans addition of the 2-amino group of guanine to the 10-position of the (+) enantiomer of the anti-diolepoxide derivative of BP. This finding contributes to basic understanding of the mechanism whereby environmental exposure to BP and other polycyclic hydrocarbons leads to causation of cancer in humans.

Animal Resource Activities

Harlan Industries, Inc. (N01 CP 55647) is producing AxC inbred and Sprague-Dawley random-bred strains of aged male rats for use in prostate cancer research.

CONTRACT NARRATIVES

INTERDISCIPLINARY PROGRAMS AND RESOURCES OPERATIONAL UNIT

October 1, 1977 through September 30, 1978

CHICAGO, UNIVERSITY OF (NO1-CP-33385)

Title: Synthesis of Derivatives of Carcinogenic Polycyclic Hydrocarbons

Contractor's Project Director: Dr. Ronald G. Harvey

Project Officer (NCI): Dr. David G. Longfellow

Objectives: To synthesize polycyclic hydrocarbon derivatives and metabolites to be made available by NCI to the scientific community for in vivo and in vitro studies of carcinogenesis, mutagenesis, and related processes. These compounds are of special value in studying enzyme pathways in carcinogenesis, and certain of these compounds are implicated as the active forms of the carcinogenic hydrocarbons.

Major Findings: In the initial phase of this program syntheses were developed for virtually all known or suspected oxidized metabolites (phenols, quinones, dihydrodiols, arene oxides, diolepoxides, etc.) and related isomeric compounds derived from the carcinogenic hydrocarbons benzo[a]pyrene (BP) and benz[a]anthracene (BA). Work in the BP and BA series is currently nearing completion. Studies in progress are principally directed towards exploratory and preparative scale syntheses of analogous metabolites and related derivatives of chrysene, benzo[e]pyrene (BeP), dibenz[a,c]anthracene (DBa,cA), 7-methylbenz[a]anthracene (7-MBA), 7,12-dimethylbenz[a]-anthracene (DMBA), triphenylene, and 3-methylcholanthrene (3-MC).

In view of the strong evidence implicating certain diolepoxide derivatives as the ultimate active forms of carcinogenic hydrocarbons, synthesis of these compounds has been assigned highest priority. Small scale exploratory syntheses of one or more dihydrodiols and the corresponding anti-isomeric diolepoxide derivative of each of these hydrocarbons, excepting 3-MC, have recently been achieved. Synthesis of the oxidized derivatives of the alkylated hydrocarbons (i.e., 7-MBA, DMBA, 3-MC) via methods directly analogous to those devised previously for the BP and BA compounds has proven not practically feasible, and alternative synthetic methods involving novel synthetic principles have had to be developed. Improved synthetic approaches to the hydrocarbons BeP and DBa,cA have had to be developed. Improved synthetic approaches to the hydrocarbons BeP and DBa,cA have also been successfully carried to completion, making these relatively rare and expensive polycyclics available in sufficient quantities as starting materials for all projected studies.

Significance to Biomedical Research and the Program of the Institute: Recent evidence supports the theory that metabolic activation is essential to the carcinogenic activity of the polycyclic aromatic hydrocarbons. Specifically, the (+)-anti-diolepoxide of BP has been shown to be exceptionally mutagenic, carcinogenic and to be the principal metabolite of BP bound in vivo to the DNA of mouse, bovine, and human cells. Preliminary studies from this and other laboratories support the intermediacy of the analogous diolepoxide derivatives, particularly those in bay molecular regions, as the ultimate active forms of BA, BeP, 7-MBA, DMBA, chrysene and 3-MC. When preparative scale syntheses of these and other compounds synthesized under this program are completed the compounds will be submitted to the Chemical Repository at IITRI and made available to qualified investigators for carcinogenesis research. Due to the complexity of the synthetic methods and the low stability of many of the intermediates it would be virtually impossible for biomedical investigators lacking specialized experience in this field to synthesize any one of these compounds. As a direct result of this program, carcinogenesis research is feasible which could not otherwise be carried out. This has proved to be one of the most productive and exciting areas of current carcinogenesis research.

Proposed Course: Priority will be given to completion of exploratory experiments to develop syntheses of the compounds most urgently required for carcinogenesis research. Those compounds, particularly the dihydrodiols and diolepoxides, for which satisfactory synthesis have been devised will be prepared on sufficient scale for submission to the Repository. Investigation of potential synthetic routes to other oxidized metabolites of these polycyclic hydrocarbons will continue.

Date Contract Initiated: June 20, 1973.

Current Annual Level: $111,300

CHILDREN'S HOSPITAL OF PHILADELPHIA (N01-CP-65803)

Title: Oncogenesis and Other Late Effects of Cancer Therapy

Contractor's Project Directors: Dr. Anna T. Meadows

Project Officer (NCI): Dr. Curtis C. Harris

Objectives: The objectives of this study are to determine the magnitude and nature of the late sequelae of treatment for childhood cancer utilizing data available from 11 major pediatric cancer centers in the U.S., Canada and Western Europe. These sequelae include second malignant neoplasms (SMN) as well as nononcogenic deleterious late effects relating to organ dysfunctions later in life. This study will also determine the etiologic relationships between treatment (surgery, radiation and chemotherapy) and those sequelae observed.

Other (non-oncogenic) late effects are studied by surveying the status of 5 year survivors at each of the 11 pediatric centers for specific organ dysfunctions related to treatment with ionizing radiation and chemotherapeutic agents. Single institution in-depth studies are also underway, such as the contractor's own study of changes in intellect and learning ability resulting from radiation to the cranium along with intrathecal and systemic drug therapy for acute lymphocytic leukemia (ALL).

Major Findings: Oncogenic Late Effects: To date, a total of 150 new tumors in children with a first neoplasm have been reported from the 11 participating institutions and can be summarized as follows: radiation-associated without genetic disease, 77; radiation-associated with genetic disease, 22; non radiation-associated without genetic disease, 30; and non radiation-associated with genetic disease, 21. Of the 26 retinoblastomas included in those with genetic disease, 15 were associated with XRT. Of interest are the following: (a) The proportion of SMNs associated with radiation appears to be constant, (b) Chemotherapy is still not seen as a major factor in the etiology of these SMN, (c) Two Hodgkin's disease patients are noted with cancer of the thyroid in irradiated sites whereas this combination had not been noted previously, (d) Genetic susceptibility to raidation-associated SMN is suggested in von Recklinghausen's disease by a child who developed a schwannoma and an osteosarcoma in the area irradiated for a rhabdomyosarcoma.

Non-Oncogenic Late Effects - Survey of long term survivors: An intensive review of the charts of 120 surviving patients at the Children's Hospital of Philadelphia diagnosed in the years 1968 through 1972 revealed one Wilms' tumor patient and one neuroblastoma patient with life-threatening pulmonary restrictive disease who were treated with large volumes and doses of irradiation to the chest. Six severe late effects in rhabdomyosarcoma patients involve 4 patients; one of the two with pelvic exenteration has had multiple episodes of intestinal obstruction after large doses of irradiation to the bowel. This review enabled the contractor to design an abstract form which can be coded and key-punched directly to be used for the 11 institution survey of 5 year survivors. It permits calculation of the magnitude of the problem of late effects and will signal hitherto unexpected toxicities from new combinations of therapy, especially those resulting from treatment with multiple agent chemotherapy.

Neuropsychologic Studies: The contractor tested 21 children with ALL, all of whom received cranial irradiation, using standard intelligence tests and projective devices prior to radiotherapy and 16 were re-examined one year later. Controls were 5 children with Wilms' tumor examined before and after treatment who received no cranial irradiation but who did receive vincristine, a peripheral neurotoxin. Preliminary results suggest that consistent, cognitive deficits associated with short term memory and visual-motor perceptions are present, with younger children being at greatest risk. Nine of the 16 retested children, with ages at diagnosis ranging from 3-8 years (med. 4 yrs.) obtained lower scores on standardized intelligence tests one year later with most showing diminished attention span and particular difficulty in performing perceptual motor tasks.

Those showing no changes ranged in age from 3-10 years (med. 6 years). All Wilms' tumor patients retested showed no changes and were 5-13 years (med. 6 1/2 yrs.) at diagnosis.

Significance to Biomedical Research and the Program of the Institute: An understanding of the delayed consequences of treatment for cancer in childhood is important in the planning of therapy protocols and in counseling patients effectively. In addition, a study of the patients developing second cancers may contribute to an understanding of carcinogenesis by isolating mechanisms and risk factors in oncogenesis.

Proposed Course: Oncogenic Late Effects: The data will continue to be collected and analyzed for etiologic factors and for the incidence of SMN in children. Risk factors such as chemotherapy related to the development of SMN will also be studied by a new case control study. Pathology of SMN and radiation dose response relationships are continuing to be studied.

Non-Oncogenic Late Effects: The five-year-surviving cohorts of patients culled from the Late Effects Study Group institutions will be surveyed for those late effects which are clinically obvious and disabling, or even cosmetically severe. This will be done so that the overall magnitude of the problem of late effects can be detected by monitoring a large population of survivors who have been treated with multiple agents, surgery and irradiation.

Additional surveys of large populations of surviving children such as those entered in the NWTS will be made as an outgrowth of this study and it is expected that the other Intergroup Studies will follow suit.

Date Contract Initiated: October 1, 1977

Current Annual Level: $138,000

COLUMBIA UNIVERSITY (NO1-CP-23234)

Title: Development of a Tissue Culture Transformation System for Aromatic Amine Carcinogens

Contractor's Project Director: Dr. I. Bernard Weinstein

Project Officer (NCI): Dr. Stuart S. Yuspa

Objectives: The overall goal of this contract is the development of epithelial cell systems which will be useful as a rapid assay system for environmental agents that may cause cancer and for studies on the mechanism of chemical carcinogenesis. Emphasis is placed on the interaction between multiple factors in the carcinogenic process.

Major Findings: A prerequisite for in vitro studies on epithelial cell cultures is the development of reliable markers of malignant transformation. In collaborative studies with R. Montesano and K. K. Sanford four parameters were evaluated in 22 tumorigenic and nontumorigenic rat liver epithelial cell cultures, cytology, growth in agar, production of plasminogen activator (PA), and tumorigenicity. It was found that cytologic diagnosis and growth in agar were reliable means of predicting the malignant potential of these cultures; plasminogen activator production, however, did not correlate with tumorigenicity or metastatic potential. Related studies indicated that PA production was a more reliable predictor of tumorigenicity of transformed hamster embryo and guinea pig fibroblast cultures than for epithelial cultures. Alterations in cell surface fucose-labelled glycopeptides were demonstrated in transformed epithelial cell cultures, and in a temperature sensitive mutant of transformed rat liver epithelial cells when grown at the permissive temperature.

In previous studies it was found that glucocorticoids are potent inhibitors of plasminogen activator production in certain transformed epithelial cells, thus providing the first evidence for hormonal control of this marker. During the past year it has been observed (in collaboration with T. J. Slaga) that when a series of antiinflammatory steroids were assessed there was a good correlation between this in vitro effect and the ability of these agents to inhibit phorbol ester-induced tumor promotion on mouse skin. These studies provide insights into new approaches to inhibition of carcinogenesis.

Since the carcinogenic process is often multifactor in its etiology and multistep in its evolution this contractor has studied the interaction of chemical carcinogens, tumor promoters and oncogenic viruses in rodent and human epithelial and fibroblast cell culture systems. It was found that the phorbol esters and certain related macrocyclic plant diterpenes are potent inducers of plasminogen activator production. These agents induce other phenotypic changes in normal cells that mimic those seen in cells transformed by chemical carcinogens or oncogenic viruses. It was also found that these agents are potent inhibitors of terminal differentiation of erythroleukemia cells, neuroblastoma cells and murine melanoma. Both effects are maifest by compounds that are active as tumor promoters on mouse skin but not by structural analogs that are inactive as tumor promoters. These results provide insight into the mechanism of action of this class of tumor promoters and may provide rapid in vitro screening tests for potential tumor promoters in the human environment. Further, the phorbol esters enhance in vitro transformation of cells previously exposed to an adenovirus, or to adenovirus plus polycyclic aromatic hydrocarbon carcinogens. The latter results provide an in vitro model for studying the interaction between multiple types of etiologic factors in the carcinogenic process.

Significance to Biomedical Research and the Program of the Institute: 1) Since over 80% of human cancers arise from epithelial tissues, it is important to study the action of carcinogens on epithelial cells in

culture. 2) Since carcinogenesis may be multifactor in its etiology, it is essential to obtain information on the in vitro effects of both initiating agents and tumor promotors.

Proposed Course: Studies will be continued on: 1) the interactions between multiplefactors on in vitro transformation of epithelial cells and 2) the biologic and biochemical effects of tumor promoting agents in cell culture systems.

Date Contract Initiated: April 1, 1972

Current Annual Level: $62,094

DOE-NCI INTERAGENCY AGREEMENT OAK RIDGE NATIONAL LABORATORY (Y01-CP-50200)

Title: NCI-DOE Carcinogenesis Program

Contractor's Project Director: Dr. R. J. Michael Fry

Project Officer (NCI): Dr. Ira Kline

Objectives: The broad objective of the program is to provide an interdisciplinary research approach to solving problems of chemical carcinogenesis. This includes research on the relationship of cell membrane transport to regulation of cell growth, DNA repair, metabolism and activation of chemical carcinogens, in vitro carcinogenesis, and respiratory carcinogenesis. Thus, these studies extend from the molecular level to the whole animal. Considerable progress has been made toward establishing an interdisciplinary approach to the overall problem of chemical carcinogenesis. However, for the purpose of emphasizing the major findings and significance of the various research components, they are presented individually in this report.

Date Contract Initiated: January 14, 1963

Current Annual Level: $1,171,420

1. Repair Mechanisms in Carcinogenesis: Membrane Tunover

Specific Project Director: Dr. John S. Cook

Project Officer (NCI): Dr. Tsuyoshi Kakefuda

Objectives: Transformed cells show enhanced transport of small metabolites, and in some cases the safety factor of transport over growing cell's requirements may be so low as to approach being rate-limiting. The area of current concern is how the changes in transport properties are related to the regulation of metabolism in the transformed cell and how changes in transport properties are related to the regulation of growth in

both transformed and nontransformed cells. Of ultimate concern are mechanisms whereby development of the transformed phenotype represents modification or faulty regulation of normal physiological processes such as the induction of transport systems or the turnover of membrane enzymes. Much of the work with cultured human cells in this laboratory has been done with HeLa S3 or a diploid skin fibroblast HSWP. For controllable transformation systems, work has recently been initiated with hamster cell lines derived from BHK. These lines are temperature-sensitive for transformed functions, and have been chemically transformed with nitrosomethylurea (NMU), 4-nitroquinoline oxide (4-NQO), or dimethylnitrosoamine (DMN). With such cells, growth at the permissive temperature yields a tumorigenic population, whereas growth at the nonpermissive temperature yields a normal population; direct comparative studies can be made on the membrane properties of genetically identical tumor and normal cells.

Major Findings: (1) Amino acid uptake by cells is coupled to Na movement. Na is concentrated outside cells and tends to move in, carrying with it amino acids like the analog AIB (α-aminoisobutyric acid). A major force driving Na-coupled AIB is the electrical potential across the membrane; this increases substantially with growth and allows for a markedly enhanced transport of amino acids. The effect of this change is an increase in the apparent affinity (decrease in K_m) of the transport system for its substrate. This laboratory has derived a model to explain how these factors interact, and has successfully tested the model in two systems. First, with the NMU, 4-NQO, or DMN chemically transformed BHK cells growing at their permissive (transformed) temperature, the accumulation of AIB is consistent with the enhanced membrane potential associated with unregulated growth. The second system is Friend erythroleukemic cells treated with dimethylsulfoxide (DMSO); they slow their growth and differentiate into hemoglobin producing cells. This effect can be prevented with the tumor promoter tetradecanoyl-phorbol-acetate (TPA). Furthermore, when the potential is estimated by the AIB accumulation ratio, it is found to be reduced when growth is inhibited and differentiation is induced with DMSO. Conversely, growth is maintained and the potential actually enhanced, even in the presence of DMSO, when the cells are treated with tumor promoter. It has been provisionally concluded that the membrane potential is an important concomitant and possibly even causal factor in growth regulation. (2) The uptake and metabolism of glucose is markedly altered in malignantly transformed cells. Three aspects of this problem have been investigated: (a) The rapid glucose metabolism of tumor cells can be controlled by the flavonoid compound quercetin, which was thought to act on a specific intracellular enzyme. It has now been shown that quercetin (and related compounds) act at the first step, i.e., entry of glucose into the cell. The chemical form that is effective has been established and it has been shown that quercetin acts by intercalating in the lipid bilayer of the membrane, possibly increasing its local rigidity. (b) Glucose transport is enhanced as a concomitant to malignant transformation in the temperature-sensitive, chemically transformed hamster (BHK) cells. The first intracellular enzyme to act on the transported glucose is hexokinase. It has been found that hexokinase is membrane-bound in the transformed cells, and studies continue to determine

whether this membrane-bound form is subject to the usual regulatory factors. (c) During induction of enhanced glucose transport in human cells, there is co-induced a glucose-6-phosphatase activity that is increased as much as 10-fold over normal controls.

Significance to Biomedical Research and the Program of the Institute: Current thinking about tumorigenesis emphasizes the altered state of surface membrane. The alteration includes enhanced capacity for the uptake of metabolites, either because the transformed cells have a greater number of transporters or the activity of existing transporters is increased, or both. The program described here is concerned with (a) the mechanisms of enhanced transport and their relations to the actions of certain tumorigenic or other growth-regulating chemicals, and (b) the physiological interactions of transport, metabolism and the regulation of growth.

Proposed Course: The role of the membrane potential as a growth regulator will be pursued in detail. The Friend cell system, where logarithmic growth vs. hemoglobin production offer positive criteria for growth vs. nongrowth, and where effects of tumor promoters are readily assessed, will be further developed. The chemically transformed BHK cells will also be used for a similar analysis. Ionophores shall be exploited to experimentally manipulate the potentials and thereby influence growth regulation. In physiological experiments attempts shall be made to identify the ionic mechanisms underlying the growth-associated potential changes.

Studies on physiological regulators of glucose transport, hexokinase, and glucose-6-phosphatase will be continued, using the temperature-sensitive BHK cells. Attempts to define the proximate inducer(s) of transport and of glucose-6-phosphatase will be made. There is concern whether the latter induction is specific or represents a generalized increase in activity of the endoplasmic reticulum. If the latter turns out to be the case, the contractor shall explore whether this activity leads to a generalized increase in turnover and modification of surface enzymes associated with malignant transformation.

Date Project Initiated: March 1, 1976

2. DNA Repair Mechanisms in Chemical Carcinogenesis

Specific Project Director: Dr. James D. Regan

Project Officer (NCI): Dr. Rufus Day

Objectives: The major objectives of the DNA Repair Program are to define and characterize the various kinds of prereplication and postreplication DNA repair that occur in human cells after treatment with representative carcinogens and metabolites of carcinogens, in an effort to understand

the relationship between the lesions induced in DNA, their alteration by repair enzymes, and the ultimate consequences of these events.

Major Findings: DNA Damage and Repair Caused by Safroles. Among a series of eight safrole derivatives (supplied by Drs. James A. and Elizabeth Miller, University of Wisconsin), the most electrophilic of this series, 1'-acetoxysafrole, produced a typical UV-type repair response in normal human cells. Approximately 50 nucleotides were patched into the average repaired region of DNA after treatment with this compound. However, experiments with xeroderma pigmentosum cells (expected to be defective in repair after treatment with this compound) showed normal repair. These results suggest that the damage induced by the 1'-acetoxysafrole is not a UV-type damage recognized by the UV-endonuclease system of human cells. A study has been completed of UV-endonuclease-sensitive sites induced in human cells by 1'-acetoxysafrole compared to cyclohexylcarbamate (a typical UV-type chemical carcinogen). While cyclohexylcarbamate induced many UV-endonuclease-sensitive sites in the DNA of human cells, typical of a UV-type agent, no such sites were introduced in the DNA by 1'-acetoxysafrole. This result is consistent with previous data from this laboratory showing that xeroderma cells repair this damage normally. Thus, it is believed with 1'-acetoxysafrole that a heretofore undescribed DNA repair system is being observed. That system is currently under investigation.

DNA Repair in Human Colon Cells. A series of human colon carcinoma cultures were examined for their ability to repair UV and chemical damage to their DNA. Extensive bromodeoxyuridine photolysis assays both with UV-induced damage and damage induced by n-acetoxyacetoaminofluorene or bischloronitrosourea have confirmed that in all the colon carcinoma cell lines there is a reduction of 50 to 70% of normal repair as compared to the human cells most generally studied, i.e., fibroblasts. Group 1 and 2 adenocarcinomas showed 30% of normal repair following UV radiation. Group 1 cells were also sensitive to hydroxyurea resulting in a reduction in repair to about 20% of normal skin cells. Group 1 cells were 50% defective in repair of n-acetoxyacetoaminofluorene damage whereas Group 2 cells were comparable to normal skin cells. Both groups showed photosensitivity to BCNU and evidence for an x-ray type of damage which was not defective and was comparable to normal human fibroblasts. Additional studies with normal colon cell lines are necessary to determine the significance of these results.

Effect of Hydroxyurea on Human DNA Repair. There have been several conflicting reports in recent literature concerning the inhibitory effect of hydroxyurea (HU) on repair of UV-induced DNA damage. Since HU may inhibit the repair process, an extensive investigation into HU and its possible inhibitory effect on excision repair systems in humans has been completed. In this study the bromodeoxyuridine photolysis assay has been utilized with and without HU, direct measure of thymidine dimers, and measurement of UV-endonuclease-sensitive sites of DNA. Results so far indicate that a small amount of inhibition (0-15%) of DNA repair does occur with a concentration of 2mM HU. At higher concentrations (10 mM)

however, considerable inhibition (up to 70%) of repair processes occur. Along with this inhibition there appears to be a concurrent increase in size of the average repaired region. The accumulation of DNA strand breaks with HU reported by other investigators has not been seen.

Significance to Biomedical Research and the Program of the Institute: In all experiments involving the treatment of cells or organisms with chemical carcinogens, repair of the lesions induced by the agents is a significant chemical event and should be investigated. It is conceivable that carcinogenesis may occur as a result of residual, i.e. unrepaired lesions, in the DNA. In this institute, extensive experiments involving treatment of cells, animals, and microorganisms with chemical carcinogens are being carried out. Thus it is most essential that there be, as an integrated part of this program, a laboratory working exclusively on DNA repair after treatment with chemical carcinogens.

The significance of these studies lies in: 1) The ubiquitousness of repair (most organisms including man have several complex repair systems); 2) the belief that mutagens and carcinogenic events may arise only from residual (nonrepaired) lesions or that error-prone repair systems may be the major induction mechanisms of the mutagenic or carcinogenic event; 3) the clear association of repair defects and highly carcinogenic disease states in man (Xeroderma pigmentosum).

Proposed Course: To continue experiments on prereplication repair using selected carcinogens and their metabolites and to determine in molecular terms the repair mechanisms already found operative with certain carcinogen metabolites. In postreplication repair, this laboratory has previously reported on the effect of Rauscher Leukemia Virus in reducing postreplication repair in rat cells. Rauscher Leukemia Virus temperature sensitive mutants are now being studied in mouse cells in an attempt to find a relationship between the temperature sensitive mutants and the effect on postreplication repair.

Date Project Initiated: October 1, 1974

3. Mechanism of Action of Chemical Carcinogens

Specific Project Director: Dr. James K. Selkirk

Project Officer (NCI): Dr. Andrew C. Peacock

Objectives: The major objectives of the program on mechanism of action of chemical carcinogens are to study both tissues and cells of susceptible and resistant species that more closely approximate human carcinogenesis and to investigate the metabolism of polycyclic carcinogens. Metabolism of carcinogens is investigated in terms of metabolite profile, kinetics of activation and detoxification, isolation and identification of active carcinogenic intermediates and elucidation of the differences between carcinogenic and non-carcinogenic isomers of polyaromatic molecules.

This includes formation and half-life of the active carcinogenic species, quantitation of binding to cellular marcomolecules and isolation of bound adducts; development of innovative analytical methods to isolate and identify the activated macromolecular species and detoxification products of several types of chemical carcinogens.

Major Findings: A number of rodent and human cells and tissues were studied in order to differentiate the preferential pathway followed by resistant and susceptible cells toward malignant transformation by benzo(a)pyrene. A number of parameters were observed that dictate how the carcinogen will affect the cell or tissue. It has become clear that activation and detoxification require several enzymatic steps that will vary according to the conditions under which the experiment is carried out. It has been shown that microsomal incubations from rat, mouse, and hamster liver and tissue culture cells produce a number of derivatives as final products which are not found when the carcinogen is incubated with intact cells. This is due to the fact that disruption of the spatial orientation of the enzyme complex within the cytoplasmic membrane destroys the programmed order of metabolism which begins as an epoxide intermediate followed by hydration to the corresponding diol, followed by conjugation to glucuronide, glutathione or sulfate for excretin from the cell. Accumulation of phenolic products which are weak acids, increases the cytotoxic effect and may indeed alter the ability of the cell to maintain its normal physiology. It has been determined that primary fibroblasts exhibit insigificant amounts of 3-hydroxy and 9-hydroxy benzo(a)pyrene indicative of active glutathione and glucuronide transferases. In addition, there are increases in the 9,10-dihydrodiol over long-term incubation in all fibroblasts and epithelial cells, suggesting this metabolite is a poor substrate for both re-metabolism to diol-epoxide or for conjugation by cellular transferases. This metabolite may indeed be a metabolic end-product. A study using rat liver epithelial cell lines developed at the International Agency for Research on Cancer in Lyon, France (Dr. R. Montesano) has been completed. The parent non-malignant line (IAR-20), along with DMN (IAR-28), and MNNG transformed lines (IAR6-1) were used. Studies to discern in a comparative fashion the perturbation in the enzymatic activation steps between the parent and the transformed lines have been performed. Both normal and transformed clones yielded identical qualitative metabolite profiles suggesting that activation and detoxification pathways continue to favor the same regions of the benzo(a)pyrene molecule. No hitherto undiscovered metabolites were found. Major variations appeared in the ability of the cell lines to turnover benzo(a) pyrene. The transformed lines exhibited a greatly depressed capability for induction by benz(a)anthracene. This effect was further seen by reduced cytotoxicity in the transformed line.

The ability of human organ culture material to activate benzo(a)pyrene has been studied in collaboration with Dr. C. C. Harris at NCI. It was found that normal, human bronchus produces significant quantities of the 7,8-dihydrodiol which is known to be a major subtrate for remetabolism of benzo(a)pyrene to a diol-epoxide. This has been followed up by showing that this tissue, in the presence of benzo(a)pyrene as a substrate, will

produce mutagenesis in the V79 hamster test system (Dr. I. C. Hsu). Most recently, in collaboration with the Environmental Protection Agency (Dr. J. Huisingh), test systems have been developed to study primary hepatocytes for benzo(a)pyrene activation and detoxification. Preliminary studies suggest that hepatocytes from rat and hamsters follow significantly different activation kinetics than fibroblastic cells, epithelial cells, or in vitro incubation with microsomes. It would appear that hepatocytes can attack both the K-region and non-K-region sides of the benzo(a)pyrene molecule and suggests different metabolic kinetics for this type of cell.

Sigificance to Biomedical Research and the Program of the Institute: Human susceptibility to chemical carcinogens is largely based on epidemiological and animal model studies. Extrapolation from tissue and animal studies to human requires the test system be as close in proximity to human biochemistry as possible. It is, therefore, critically important that activation, detoxification, and macromolecular interaction in biosystems that are routinely used for carcinogen testing, be understood and their activation pathways determined and compared. In this manner we will be better able to extrapolate in vitro and in vivo laboratory findings toward estimating human risks from exposure to suspected carcinogens.

Proposed Course: High-pressure liquid chromatographic assays for rapid and accurate determination of polyaromatic metabolites will be used. Continued studies with fibroblast and epithelial cells, both human and rodent will be utilized. Attempts will be made to determine further the major steps in the biochemical pathways between resistant and susceptible cells in tissues. These studies will be broadened to encompass isomer pairs of benzo(a)pyrene and dibenzanthracene in which one isomer is highly carcinogenic and mutagenic and the other isomer is negative. In this manner a decision can be made as to which active intermediates are the preferential alkylating agents for cellular macromolecules. With regard to the latter, isolation and study of DNA, RNA and protein from susceptible and resistant cells which have been reacted with carcinogenic and non-carcinogenic isomers has been started. It is planned to determine both quantitative and qualitative variation for the isomer pairs in resistant and susceptible cell systems.

Date Project Initiated: March 1, 1976

4. Respiratory Carcinogenesis

Specific Project Director: Dr. Paul Nettesheim

Project Officer (NCI): Dr. Carl E. Smith

Objectives: To analyze co-carcinogenic effects of physical and chemical agents in the induction of lung cancer; to identify and study those environmental agents which may increase the susceptibility of respiratory tissues to normally ineffective doses of carcinogen; to develop new

experimental models for cofactor studies; to study host factors that affect tumor induction and tumor progression; to identify the major steps in the evolution of respiratory tract cancer using in vivo, in vivo-in vitro, and in vitro approaches and to uncover early indications (markers) for preneoplasia; to investigate the inhibitory effects of retinoids on the development of respiratory tract neoplasia both in vivo and in vitro.

Major Findings: (1) The study of the evolution of neoplasia in tracheal transplants after exposure of 165   of DMBA over a 4 week period showed (a) changes in the respiratory epithelium of first a general squamous metaplasia, (b) replacement with a "normal-looking" epithelium, (c) the appearance of focal lesions at 3-4 months, (d) a decrease in the total number of focal lesions at 4 to 12 months, but an increased atypia in the remaining lesions. The final tumor incidence remains to be determined.

(2) During the past year a series of experiments were carried out to study the carcinogenicity of nickel subsulfide and chrysotile asbestos in tracheal transplants. Nickel was a weak carcinogen for epithelium, but highly sarcomagenic when delivered in large amounts (3 mg/trachea). The asbestos was a weaker carcinogen, but caused marked irritation.

(3) During the past year, tumor promotion and co-carcinogenesis studies were initiated. In the first case, pellets containing 100 µg TPA were implanted in tracheal transplants previously exposed to 100 µg DMBA. TPA caused an intense inflammatory and toxic reaction, but after 8 months no tumors have appeared. Co-carcinogenesis studies with (BaP+BeP) and with (DMBA + asbestos) are in progress.

(4) A study was initiated to test the effect of carcinogen on aged tissue. Tracheas from 12-week-old and 24-month-old rats were transplanted into young host and exposed to 100 or 150 µg DMBA. Tumor incidence at 12 months indicates no preferential susceptibility. This study is still in progress.

(5) Tracheal tumor induction studies in hamsters with N-nitrso-N-methylurea (NMU) delivered with a specially designed tracheal catheter were completed. The effect of frequency of exposures (10-30 exposures) and NMU concentration (0.25-1.0%) on tumor incidence, tumor type and tumor latency were established. Tumor incidences from 20-90% can be achieved depending on the exposure modality chosen with tumor induction times of 10-50 weeks. At low NMU concentration, adenocarcinomas outnumber other histological tumor types.

(6) A study of the effects of two different retinoids on tracheal tumor induction with NMU is coming to a conclusion. The two retinoids were administered in the diets at maximal tolerated doses and 60% MTD beginning at the end of carcinogen exposure. Three different carcinogen exposure levels were chosen to induce carcinoma incidences as low as 55% and as high as 80% in animals on control diet. To date, none of the retinoid treatments appear to produce any significant inhibition of tumor development.

(7) Evaluation of the past inhalation co-carcinogenesis studies provided no conclusive evidence for the co-carcinogenicity of inhaled nitrogen dioxide, formaldeahyde and acrolein.

(8) Immunological studies were carried out with a number of respiratory tract carcinomas induced in rats and mice by topical application of polycyclic hydrocarbons. These studies showed that the majority of the carcinomas are demonstrably antigenic, that many of them have cross-reacting antigens which are neither of embryoneal or of viral origin and that their antigenicity increases markedly when they are grown in vitro for extended periods of time.

(9) An in vivo-in vitro experiment to test whether tracheal epithelium exposed to carcinogen (150 μg or 640 μg DMBA) in vivo over a relatively short time (2 weeks) would show differences in growth behavior, and if and when oncogenic transformation would appear after propagation of the cells in vitro, was completed. Some dose-related effects were observed. a) More primary cultures and cell lines could be established from tracheas exposed to the high DMBA dose. b) Differences were seen in the time cells were maintained in vitro before becoming oncogenic: in the 150 μg group it was 400 days or longer, and in the 640 μg group it was 200 to 400 days. c) Tumor induction times were shorter (9 to 60 days) for cell lines with short in vitro "latency" periods and long (100-250 days) for cell lines with long in vitro latency. Reliable phenotypic markers for oncogenicity in this system were growth in soft agar and high colony-forming efficiency on plastic.

(10) In vivo-in vitro experiments designed to search for quantitative and qualitative changes presumed to occur in vivo after carcinogen exposure are in progress. Epithelial cells were stripped 0, 8, 16, and 32 weeks after exposing tracheal transplants to 100-150 μg DMBA over a 4-week period. The number of these cells that showed an enhanced growth capacity in vitro as well as th e fraction of altered cells isolated which yielded tumors after inoculation in vivo increased with time. Other quantitative and qualitative changes are being assessed.

(11) A new tracheal repopulation technique has been developed to test the differentiative and oncogenic capacity of epithelial cells. Tracheas with intact, partially or totally denuded epithelium which are repopulated with normal cell suspensions exhibit a normal mucociliary epithelium. Preneoplastic and neoplastic cell lines invaded the surrounding tissues. The presence of normal epithelial cells forms an effective, but not absolute, barrier to neoplastic cells.

(12) An organ culture-cell culture system was used in the past 2 years to demonstrate in vitro oncogenic transformation of respiratory epithelium by exposure to N-methyl-N-nitro-N-nitrosoguanidine (MNNG). Now, multistage carcinogenesis was also demonstrated in this system by exposing organ cultures to 12-O-tetradecanoyl phorbol-13-acetate (TPA) after MNNG exposure. Tumor promotion was seen in terms of increase in the frequency of transformation and decrease in the time at which oncogenic transformation was

observed. It was also demonstrated that TPA, alone, can permanently alter the growth regulating mechanisms of epithelial cells. Cell lines could be derived from TPA-exposed tracheal organ cultures similar to those obtained from carcinogen-exposed organ cultures. However, in contrast to the carcinogen-exposed cell lines, the TPA cell lines are not tumorigenic. Cell lines could not be established from control organ cultures.

(13) Keratinizing epithelial cell lines, established from DMBA-exposed tracheas, are being effectively utilized to study the active and toxic levels of retinoids in vitro at the target cell level.

(14) Preliminary experiments investigating the possible oncogenicity of radiation to tracheal epithelial cells have been carried out. A dose of 600 rads X-ray delivered to a non-tumorigenic but "initiated" cell population decreased the time to oncogenicity by 80 days. Thus far, x-radiation has not been found to be oncogenic to normal tracheal epithelium.

Significance to Biomedical Research and the Program of the Institute: Epidemiologic data indicate a multifactorial etiology in human lung cancer. This work is a study, using animal models and in vitro systems, of the role of chemicals, and of physical and biological agents influencing the respiratory tract in the development of lung cancer. It is expected that this approach will help to define the decisive environmental and host factors in the pathogenesis of lung cancer. The studies are also aimed at elucidation of the evolution of lung cancer, particularly the early preneoplastic phases, in order to identify the essential steps in the development of this neoplastic disease.

Proposed Course: (1) To continue the study of chemical carcinogenesis in respiratory epithelium with the tracheal transplant system. (2) To conduct co-carcinogenesis studies with the tracheal transplant system. (3) To continue the study of neoplastic transformation of respiratory epithelium using the various in vivo-in vitro and in vitro culture systems developed in this laboratory. (4) To continue to develop indicators and markers for preneoplasia and neoplasia for respiratory epithelium. (5) To continue the in vitro studies of the effective and toxic levels of retinoids in keratinizing epithelial cell cultures. (6) To initiate studies of the mechanism of action of retinoids on reversing keratinization using epithelial cell cultures.

FRANKLIN INSTITUTE RESEARCH LABORATORIES (N01-CP-65812)

Title: Information Support: Retinoid Program/Human Tissue Study Program

Contractor's Project Director: Dr. Marjorie Nemes

Project Officers (NCI): Dr. Curtis C. Harris
Dr. Carl E. Smith

Objectives: 1) To provide information support services to the Retinoid Program by collecting, classifying, and indexing papers concerning the effects of Vitamin A on cancer and other cellular changes as well as other Vitamin A-related topics as discussed and requested by the Project Officer. 2) To provide information support services to the Human Tissue Study Program on in vitro carcinogenesis (chemical, physical, and viral) in human tissues as cell or organ cultures and xenotransplantation of neoplastic and non-neoplastic tissues in a variety of intact in vivo host systems.

Major Findings: More than 2000 papers have been identified, obtained, evaluated, and indexed in-depth to provide background and current information in support to these two areas. Full text copies of the papers have been supplied to the respective Project Officers. The information support to the Human Tissue Study Program has been presented as a triannual bulletin sent to the scientific investigators on request. The bulletin contains complete bibliographic citations of the topics classified. A special literature compilation, complete with index, was prepared for Dr. Harris for use at the Workshop on Carcinogenesis in Human Tissues held in August 1977, at Aspen, Colorado. Work has continued updating the literature review carried on last year for both areas of interest.

Significance to Biomedical Research and the Program of the Institute: This project has provided valuable literature review and quick entry to the recent literature in these fields by means of the in-depth indexes. No other source of this useful capability exists.

Proposed Course: Program plans to continue to provide these services to assist both in-house and outside scientists.

Date Contract Initiated: June 23, 1976

Current Annual Level: $38,000

HARLAN INDUSTRIES, INC. (NO1-CP-55647)

Title: Development of Colonies of Aged Rats

Contractor's Project Director: Mr. Hal P. Harlan

Project Officers (NCI): Mr. Clarence Reeder
Dr. Carl E. Smith

Objectives: The objective of this contract is to breed and age AxC inbred (ACI maintained by Alton Ochsner Medical Foundation) and Sprague-Dawley random-bred strain rats for the Carcinogenesis Program. Males of both species are placed in a barrier facility for aging purposes.

Major Findings: The project is progressing well, and contractor is now into the third phase of the project and is aging both Sprague-Dawley and ACI strains of rats in barrier colonies. The oldest animals inhouse are 27 months of age, with normal expected survival.

Significance to Biomedical Research and the Program of the Institutes: During the latter period of life in men and in animals, a number of physiologic alterations occur which are thought to be significant events in the carcinogenic process. One of the cancers thought to be influenced by age is the induction of prostatic cancer, as judged by experimental evidence developed in the Carcinogenesis Program over the past three years. These animals will be available, at various ages, to permit experimental verification and analysis of this phenomon.

Proposed Course: To maintain these animals, to provide information to the Program on survival rates, health status, etc., and to distribute as appropriate to various investigators through the Carcinogenesis Program for prostatic and various types of aging research.

Date Contract Initiated: June 30, 1975

Current Annual Level: $210,460

IIT RESEARCH INSTITUTE (NO1-CP-65745)

Title: Preparation of Carcinogens

Contractor's Project Director: Dr. Allan P. Gray

Project Officer (NCI): Dr. David G. Longfellow

Objectives: As requested by the NCI: to supply quantities of well-characterized carcinogens, of consistent, reproducible and documented composition and purity, to the Standard Chemical Carcinogen Repository for distribution for carcinogenesis research; to provide complete information as to synthetic procedures or compound sources, physical properties, purity, analytical data; to carry out stability studies on certain of the carcinogens and furnish information as to optimum storage conditions and rates of degradation.

Major Findings: During the past year additional compounds have been prepared and supplied to the Repository and further stability studies have been carried out. A modified synthesis of N-acetoxy-2-fluorenylacetamide giving improved yields of the compound has been developed. A report on this synthesis is being prepared for publication.

Significance to Biomedical Research and the Program of the Institute: The need has been recognized by the Carcinogenesis Research Program to be able to supply researchers in carcinogenesis with well-characterized samples of carcinogens for their investigation. Samples of compounds under study should be of consistent, reproducible and documented composition and at as high a level of purity as feasible in order that investigators in different laboratories, or in the same laboratory at different times, can be confident of the nature of the material they are working with and of the validity of comparative data. One possible source of variability in research results can thus be eliminated.

Not only has it been difficult to obtain carcinogens of consistent composition from the usual commercial suppliers, but many suppliers have elected to stop handling suspected carcinogens. Thus, many of the compounds desired for research are no longer available commercially at all. Work is carried out in a toxic laboratory facility designed to meet OSHA requirements for handling carcinogens. The contractor is gaining valuable experience in the safe-handling of bulk quantities of these materials.

Proposed Course: The contract terminated on January 31, 1978. Through the auspices of a Basic Ordering Agreement the contractor will continue to synthesize and supply compounds as requested.

Date Contract Initiated: November 1, 1975

Current Annual Level: $15,000

IIT RESEARCH INSTITUTE (NO1-CP-55646)

Title: Chemical Repository

Contractor's Project Director: Dr. James N. Keith

Project Officer (NCI): Dr. David G. Longfellow

Objectives: To establish a facility for the safe storage, repackaging, and distribution of potential carcinogens for use in cancer research.

Major Findings: The Chemical Repository has completed its second full year of operation at a considerably increased level of activity. During 1977 the number of samples shipped was two thousand, or double those in the previous year. The stocks have grown from 74 compounds at the initiation of the program to 223 as of the end of the year. Many of these are chemicals transferred from surplus stocks of the Bioassay Program for preservation and for distribution to the In Vitro Program and other requestors. The repository continues to supply coded samples to designated contractors participating in the mutagenicity-carcinogenicity double-blind study being conducted under the In Vitro Program.

Numerous letter and telephone requests are answered on safe handling procedures for specific chemicals, and property data sheets are supplied with the samples of compounds shipped.

Significance to Biomedical Research and the Program of the Institute: Besides the need for effective coordination of information flow, the carcinogenesis research community has a pressing need for a centralized source of well-documented reference compounds. To this end, a chemical repository for the safe storage, repackaging, and distribution of samples for the Carcinogenesis Program has been established. This facility,

designed and operated in conformance with OSHA and EPA regulations, receives material from suppliers, repackages as required for the users, and ships samples with analytical documentation and safe handling protocols.

Proposed Course: The Repository is becoming a useful distribution center for chemicals no longer available on the market and performs a number of other service functions as well. Support activities will continue throughout the five-year contract.

Date Contract Initiated: June 29, 1975

Current Annual Level: $210,000

INFORMATION PLANNING ASSOCIATES, INC. (N01-CP-75896)

Title: Management and Technical Support Services to the Carcinogenesis Research Program

Contractor's Project Director(s): Dr. Florence Lewis
Dr. Tapas Pradhan

Project Officer (NCI): Dr. Ira Kline

Objectives: To provide management and technical support services to the Carcinogenesis Research Program of the Division of Cancer Cause and Prevention, National Cancer Institute. This entails the following: (1) To provide the Project Officer with planning and programming support (e.g., literature search, abstracts of scientific articles); (2) Furnish technical information with reports and program technical information with reports and program documentation; (3) Perform data collection, data evaluation and statistical analysis; (4) Assist in the development of long-range goals for the Carcinogenesis Research Program; (5) Provide coordination and logistical support; and, (6) Make best effort in assuming other responsibilities as desired by NCI.

Major Findings: (1) The Carcinogenesis Research Inventory covering USA funded active projects - FY 77 has been compiled. In this inventory, the research contracts and grants relative to environmental carcinogenesis have been reported as of June, 1977 and have been classified and coded according to Environmental Carcinogenesis classification scheme of the National Cancer Institute; (2) The compendium of scientists with their name, address and area of expertise has been prepared; (3) The Carcinogenesis Contract Program Management Group manuals for different meetings have been supplied to the National Cancer Institute; (4) The Annual Report of the Carcinogenesis Program, Division of Cancer Cause and Prevention, for the period July 1, 1976 through September 30, 1977 has been prepared in collaboration with NCI staff; (5) Reports of Justification for Non-Competitive Procurement (JNCP), Workscopes and State Department Clearance of

several contracts have been drafted with the assistance of NCI staff; (6) Possible participants have been listed in the Proposed Development Consultations (PDC) according to sixteen classification schemes as provided by the NCI.

Significance to Biomedical Research and the Program of the Institute: The contractor employed technical skills in the coordination and development of biomedical documentation of the carcinogenesis research program. The project was managed carefully with expert use of manpower and with high quality products at reasonable costs. The project provided the resources for satisfying information needs of investigators and science administrators in the Carcinogenesis Research Program in a timely and effective manner.

Proposed Course: It is intended to continue all the management and technical support services to the Carcinogenesis Research Program as needed. The Contractor will continue to respond to the information needs of the program and will furnish services, qualified personnel, material, equipment and facilities to perform planning and programming support, documentation, coordination and logistical support for the project.

Date Contract Initiated: April 26, 1977

Current Annual Level: $43,659

MIDWEST RESEARCH INSTITUTE (NO1-CP-65739)

Title: Preparation of Carcinogens

Contractor's Project Director: Mr. James C. Wiley, Jr.

Project Officer (NCI): Dr. A. R. Patel

Objectives: To provide adequate quantities of high purity and well-characterized reference compounds to the NCI Standard Chemical Carcinogen Repository. The project is designed to serve a dual purpose: to synthesize compounds that are unavailable from other sources, and to provide analyzed chemical standards to researchers to eliminate variability in the analysis of experimental results.

Major Findings: The carcinogen 7,9-dimethylbenz[c]acridine was prepared and shipped to the NCI repository.

Significance to Biomedical Research and the Program of the Institute: This contract provides significant quantities of well-characterized reference compounds to carcinogenesis researchers. Investigators are then able to identify and study the mechanisms of action of these carcinogenic substances and their metabolites. Information derived from this type of study is intended, ultimately, to lead to the development of methods to prevent cancer in humans.

Proposed Course: The contract terminated on January 31, 1978. Through the auspices of a new Basic Ordering Agreement the contractor will continue to synthesize and supply compounds as requested.

Date Contract Initiated: January 21, 1976

Current Annual Level: 0

MIDWEST RESEARCH INSTITUTE (N01-CP-23270)

Title: Analytical Chemistry Resource

Contractor's Project Director: Dr. Evelyn Murrill

Project Officer (NCI): Dr. David G. Longfellow

Objectives: The objective of this program is to serve as a state-of-the-art analytical facility for the Carcinogenesis Program. Its purpose is to precisely define the chemical nature of compounds to increase the accuracy and reliability of data obtained in carcinogenesis research.

Major Findings: Since June 1972, studies have been completed and reported on 245 batches of chemicals (187 different chemical substances). Ten have been analyzed in detail to identify and quantitate minor impurities, and 30 chemical mixes have been analyzed for stability or homogeneity. Routine characterization studies include chromatography (TLC, GC, HPLC, etc.), elemental analysis, water analysis, and other assay methods dependent on the chemical (titration, voltammetry, mass spectrometry). Physical data include transition data (melting point and boiling point), refractive index and optical rotation, and spectral data (infrared, ultraviolet, visible mass and NMR). Specialized tasks have included synthesis, identity and assay of pure benzo[a]pyrene; purification and assay studies of dimethyl benzanthracene; and a literature survey on the analytical methodology of the OSHA Carcinogens.

A major portion of the effort during the last year has been directed to the assay of 3-methyl cholanthrene metabolic products by high-pressure liquid chromatography, mass spectrometry and other analytical methods.

Both in vivo and in vitro liver and pancreas extracts indicated that a major site for metabolism of 3-MC is the 1-position. The methanol HPLC system did not resolve the 1-OH or 1-one from their 2-position isomers. However, the acetonitrile system did separate the position isomers and indicated that the 1-OH and 1-one were the isomers present in the samples. The cis-1, 2-diol was also present in the extract of in vivo pancreas and in vitro pancreas and liver samples.

Metabolism at the 11, 12-position appeared to be present in both the in vivo pancreas samples and the in vitro pancreas and liver samples, as illustrated by the presence of the 11-OH, the 11, 12-quinone and the cis-11, 12-diol. However, the quantity and frequency of these peaks were low compared to the 1-position metabolites.

Significance to Biomedical Research and the Program of the Institute: This contract provides the analytical expertise and instrumentation necessary to answer questions of identity or purity of chemicals used by researchers in carcinogenesis as well as provide analytical backup to assist the researcher in more sophisticated analytical investigations involving metabolic product assay and identification.

Proposed Course: The contract was completed December 31, 1977, however, analytical services will continue to be provided as requested by NCI under contract NO1-CP-33387.

Date Contract Initiated: June 28, 1972

Current Annual Level: 0

MIDWEST RESEARCH INSTITUTE (NO1-CP-33387)

Title of Project: Synthesis of Polycyclic Hydrocarbon Derivatives

Contractor's Project Directions: Mr. James F. Engel
Mr. James C. Wiley, Jr.

Project Officer (NCI): Dr. David G. Longfellow

Objectives: The overall objective of this program is the preparation of NCI-selected compounds by unequivocal methods to produce quantities of high purity and well-characterized materilas in order to allow investigators in the NCI Carcinogenesis Program, and others not directly associated with NCI, to identify products of the biochemical oxidation of polynuclear aromatic hydrocarbons. Activities in support of the NCI Standard Chemical Carcinogen Repository include supply of known and/or suspect metabolities, resyntheses, and operation of a repository for isotopically labeled derivatives (^{3}H, ^{14}C, and ^{13}C). In addition, efforts are directed toward the synthesis of compounds selected by the National Institute of Environmental Health Sciences via an interagency agreement.

Major Findings: To date, approximately 130 derivatives of the following five parent hydrocarbons have been prepared: benzo[a]pyrene, 3-methylcholanthrene, benz[a]anthracene, 7, 12-dimethylbenz[a]anthracene, and dibenz[a,h]anthracene. The products prepared thus far have included K-region and non-K-region phenols, quinones, epoxides, dihydrodiols, dihydrodiol-oxides, alkyl- and hydroxyalkyl-substituted derivatives, optical isomers, and ^{3}H-, ^{14}C-, and ^{13}C-labeled analogs.

Significance to Biomedical Research and the Program of the Institute: The elucidation of the relationship between the formation of specific metabolites of polycyclic aromatic hydrocarbons and the induction of cancer necessitates the availability of authentic samples of probable metabolities. It would be virtually impossible for a laboratory lacking specific synthetic experience to synthesize any one of these compounds

for a particular experiment. This contract allows the National Cancer Institute to provide reliable compounds for pertinent experiments in chemical carcinogenesis which could not be carried out otherwise and eliminates an important source of variability among research results. In addition, this program assures the safe handling of these dangerous materials at all steps in their processing, from synthesis, to receipt by the repository for subdivision, and ultimately to receipt by the user of a package he can open in complete confidence.

Proposed Course: Compounds will continue to be synthesized upon the request of NCI. A major effort will be devoted to the resynthesis of compounds for distribution via the NCI Repository. These will include K-region and non-K-region derivatives with emphasis on the non-K-region dihydrodiol and dihydrodiol-oxide derivatives of benzo[a]pyrene. In addition, the syntheses of similar derivatives of other parent hydrocarbons will continue, and it is expected that a concerted effort will be directed toward the preparation of ^{14}C- and ^{3}H-labeled derivatives of high specific activity.

Date Contract Initiated: June 15, 1973

Current Annual Level: $455,000

NCI FREDERICK CANCER RESEARCH CENTER (LITTON BIONETICS, INC.) (N01-CO-75380)

Title: Operation and Maintenance of the Frederick Cancer Research Center

Contractor's Project Director: Dr. Robert E. Stevenson

Project Officer (NCI): Dr. William W. Payne

Carcinogenesis Coordinator (NCI): Dr. Elizabeth K. Weisburger

Objectives: The Frederick Cancer Research Center (FCRC) Chemical Carcinogenesis Program has as a primary objective the development of a national resource capable of initiating and evaluating a broad range of approaches to effectively identify human carcinogens. To do this requires a coordinated interdisciplinary approach which consciously emphasizes the integration of basic and applied research.

Building a staff and research program into an excellent national resource is necessarily an evolutionary process which requires considerable time. As a result of the cooperative efforts of NCI staff, consultants, and the contractor's staff at the FCRC, the Chemical Carcinogenesis Program has initiated research programs in the following areas: (1) Synthesis and Analysis of Chemicals for Carcinogenicity Studies, (2) Procedures for the Safe Handling of Chemical Carcinogens, (3) Endogenous Formation of Carcinogens, (4) Endocrine Carcinogenesis, (5) Molecular Aspects of Chemical Carcinogenesis, (6) Cofactors in Chemical Carcinogenesis, (7) Mechanisms of Nitrosamine Carcinogenesis, (8) Microbial Mutagenesis, (9) Chemistry of

Carcinogens, and (10) Cellular Aspects of Chemical Carcinogenesis.

In order to provide a better understanding of the program as it is presently evolving and as it is proposed for continuation, projects indicated above are presented individually in terms of objectives, rationale, examples of results and proposed areas for continuation. Presenting them as discrete projects is simply for the purpose of highlighting the research and is not intended to imply separate and independent activity or a rigid organizational scheme. Rather, there is increasing interaction among programs, and it is the intent of the Chemical Carcinogenesis Program to foster inhouse collaboration and to extend it to include increasing collaboration between FCRC scientists and those in other institutions.

Date Contract Initiated: September 26, 1977

Current Annual Level: $4,301,794

1. Chemical Synthesis and Analysis

Contractor's Project Manager: Dr. Gary Muschik

Objectives: The objectives of this section are to provide support, expertise and methods development in synthetic and analytical chemistry for the Chemical Carcinogenesis Program (CCP) and for the interdisciplinary program at FCRC. This support and expertise includes the syntheses and analyses of labeled (^{2}H, ^{3}H, ^{14}C) and non-labeled carcinogens, their suspected metabolites, bioassay chemicals and other compounds of interest to the CCP and FCRC. These objectives are accomplished by utilizing or improving published procedures and by developing new methods in organic synthesis and analytical chemistry.

Major Findings: A new simpler method of synthesizing polycyclic aromatic hydrocarbons has been developed and applied to the synthesis of a variety of benz[a]anthracene derivatives. Several of these derivatives are model compounds used in research programs which deal with this class of carcinogens.

The development of high temperature nematic liquid crystals for gas-liquid chromatographic (GLC) separations of mixtures of polycyclic aromatic hydrocarbons and of their metabolites has continued. This procedure has been extended to the separations of bile acids and their metabolites. These procedures offer considerable advantages in the separation of some isomers which have been difficult to separate by other methods. Liquid crystal GLC phases have the potential for broad applications in analytical chemistry.

Analytical methods have been improved in the areas of thin layer chromatography separations of trace amounts of carcinogens in environmental materials, and for the separation and identification by high pressure liquid chromatography of alkylated nucleic acid bases and other materials in urine following administration of carcinogens.

Improvements have been made in the utilization of NMR spectrometry and mass spectrometry in the characterization of known and unknown compounds important to other projects at FCRC.

Significance to Biomedical Research and the Program of the Institute: The availability of this group as a resource for FCRC and for other NCI programs is important in assuring a rapid response to the immediate needs of the research programs. The availability of expertise in the area of organic chemistry provides support for biomedical projects at the molecular level in formulating ideas, and in matters of interpretation of phenomena. The development of a synthesis for unavailable carcinogens and metabolic intermediates, both isotopically labeled and nonlabeled, leads to increased efficiency in planning experiments and exploiting new leads.

Analytical support in the areas of chromatography, spectrometry, including the sophisticated techniques of mass spectrometry and NMR spectrometry leads to rapid quantitation and identification of chemicals for or from biochemical experiments. The documentation of a chemical's structure, purity, stability and homogeneous blending in a vehicle is critical for valid carcinogenicity testing. The analytical area provides this information.

Proposed Course: Work in the areas of synthesis, analysis and method development will continue. Organic chemicals of high purity, but usually in small quantities, will be synthesized to support studies of the mechanism of action of carcinogens. In many cases, isotopic labeling will be necessary.

Analytical procedures to support the bioassay project and other research areas include: physical characterization of materials by such means as UV, IR, melting point, boiling point, refractive index and the detection and characterization of impurities present; determination of the stability of substances when mixed in animal feeds, and the uniformity of mixing.

Development efforts will continue for new methods using GLC, HPLC, TLC, column chromatography and electrophoresis for isolation, detection and measurement of chemicals for studies at FCRC and for the collaboration with NCI and other carcinogenesis research facilities. The synthesis of new liquid crystals and the assessment of their suitability for separation and quantitation of carcinogens, their metabolites, and chemicals of significance to the carcinogenesis program will continue.

The provision of sophisticated mass spectrometry and nuclear magnetic resonance procedures will continue for the identification and characterization of isolated carcinogens, their metabolites and other substances of importance to the research programs at FCRC and NCI.

2. Safe Handling of Chemical Carcinogens

Contractor's Project Manager: Dr. Eric B. Sansone

Objectives: This program assists the National Cancer Institute in formulating effective measures to prevent cancers of occuptional origin in chemical carcinogenesis laboratory workers, to provide adequate protection to the general environment during the course of research programs involving the acquisition, storage, manipulation, and disposal of chemical carcinogens, and to ensure that the integrity of experimental programs is not compromised by chemical contamination.

Major Findings: Draft safety monographs have been prepared for arsenic, benzyl chloride, calcium chromate, 7H dibenzo(c,g)carbazole, dibenzo(a,i)-pyrene, lindane (γ isomer), nickel, selenium and its inorganic compounds, and styrene oxide, some of them for the Bioassay Research Section (supported under the Carcinogenesis Testing Program).

Sodium fluorescein was used as the tracer material in a study which compared the contamination in a single corridor barrier system to that in a double corridor facility when animals receive test chemical in a meal diet. More than 3,600 samples have been obtained and analyzed.

A study to determine the potential hazards associated with skin painting has been underatken. An acetone solution of the noncarcinogen, anthracene, was painted onto the backs of 175 mice. The skin painting was done in a Class II Type 2 laminar flow biological safety cabinet. A pipetting device was used for skin painting, and protocols designed to minimize contamination were followed. Samples have been collected and analyzed.

A protocol, which employs an inflatable plastic glove bag contained in a fume hood, has been devised for subdividing solid and liquid carcinogens so that contamination of personnel or equipment does not result. Five experimental runs indicate that minimal contamination results from liquid transfers. Static electricity has proved to present a problem when making solid transfers, and efforts are being made to overcome this difficulty.

An environmental monitoring program was initiated to assess employee exposure and workplace contamination associated with the production of the antibiotic daunorubicin. Sample analysis has demonstrated that worker exposures to and workplace contamination from the bacterium, media components, organic solvents and their vapors, and daunorubicin occurred.

The permeability of glove materials (natural rubber, neoprene, nitrile, polyvinylchloride, and a mixture of neoprene and natural rubber) to solutions of a number of N-nitroso compounds in water, acetone, ethanol, and methylene chloride and to several organic solvents was determined. The use of charcoal as an absorbent for contaminant vapors in air is being evaluated.

Safe methods for the destruction and disposal of chemical carcinogens are being developed using catalytic oxidation. The material to be destroyed is vaporized in a stream of air and passed over a heated catalyst bed which converts organic carcinogens to carbon dioxide, water and the highest oxidation state of any other elements in the molecule.

Significance to Biomedical Research and the Program of the Institute: Numerous laboratories throughout the world are working with chemical carcinogens. This project centralizes research and development of safety techniques for the protection of carcinogenesis research workers and the surrounding community from hazardous exposure to chemical carcinogens.

Proposed Course: To develop guidelines for safe practice by critically reviewing the relevant published and unpublished information and preparing guidelines for safe practice in handling carcinogens. To propose alternative approaches to overcome deficiencies and to investigate the most promising approaches. To serve as a resource to NCI forevaluation of safety programs in other facilities where carcinogens are handled. To investigate the contamination potential of laboratory procedures. To characterize the means and extent of chemical dissemination during various laboratory operations, to assess the risk to laboratory personnel, to the general environment, and to nearby experiments resulting from the use of chemicals. To investigate effectiveness of personal protective equipment. To investigate effectiveness of activated charcoal for the removal of gaseous contaminants. To investigate degradation, decontamination, and disposal of chemical carcinogens.

3. Endogenous Formation of Carcinogens

Contractor's Project Manager: Dr. Morris I. Kelsey

Objectives: Identification of exogenous or endogenous chemicals which may be matabolized to mutagens and/or carcinogens by enzyme systems of the gastrointestinal tract. Since chemicals of various classes frequently require metabolic activation to become mutagens/carcinogens, systems utilizing microsomal, microfloral and mucosal enzymes have been developed to study the biotransformation of a number of substrates including neutral sterols, bile acids, azo dyes, aromatic amnes and certain polynuclear aromatic hydrocarbons since they represent important chemicals encountered in our environment.

Major Findings: Microsomal enzyme studies have emphasized the role of enzyme stimulators, inhibitors, water-miscible solvents and bile acids in modifying drug metabolism. Lithocholic acid, a major human fecal bile acid, enhanced the mutagenicity in the Ames test of 2-aminoanthracene and benzo(a)pyrene when a phenobarbital-induced rat liver fraction was used. The mechanisms responsible for the metabolic activation of such different chemical carcinogens is under investigation.

The metabolism of sulfolithocholic acid (a detoxified form of lithocholic acid), lithocholic acid and taurolithocholic acid by human intestinal microflora is unique for each of the physiological bile acids. Although no mutagenic metabolites have been identified thus far, lithocholic acid (but not its sulfate ester) transforms hamster embryo cells. The possible retoxification of sulfolithocholic acid to mutagenic or new metabolites by intestinal microflora from high-risk colon cancer families is also being studied.

Cholesterol-α-epoxide, a putative carcinogen, has shown mutagen-enhancing activity under certain conditions and has transformed mammalian cells at levels comparable to 3-methylcholanthrene. The epoxide is formed from cholesterol or its palmitate ester with rat liver S_9 fractions and can be synthesized by ultraviolet light or liquid peroxidation systems. In vivo studies with mice demonstrated that the epoxide can reach the intestine independent of the route of administration and some cholestan-3β,5α,6β-triol (a detoxified metabolite) may be present.

A number of environmental azo dyes were not mutagenic in the Ames assay either in the presence or absence of a metabolic activation system. However, metabolism of these dyes by human intestinal bacteria has produced mutagenic metabolites. The development of a cell-free extract has resulted in rapid biotransformation of the dyes and facilitated the identification of certain mutagenic aromatic amine products.

Treatment of Sprague-Dawley rats with the carcinogen, 2-aminoanthracene, resulted in early inhibition of DNA and protein synthesis in liver, small and large intestine. RNA synthesis was reduced to negligible values in the colon. Baseline values of intestinal mucosal isoenzymes as possible preneoplastic markers have been obtained for studies with intestinal carcinogens. Intestinal DNA damage and repair will be studied both in vivo and in vitro as criteria for organotropic specificity of chemical carcinogens and bile acids.

Significance to Biomedical Research and the Program of the Institute: The metabolic activation of carcinogenic or co-carinogenic precursors is an important area for understanding the formation of endogenous carcinogens in humans. Studies of metabolism with enzyme systems from various portions of the digestive tract will assess organotropic effects of *in situ* formation of carcinogens, and bacterial mutagenesis evaluation of products will assist in the rapid identification of compounds which may pose an environmental hazard to man.

Proposed Course: Investigate the role of host tissue enzymes in activation and detoxification of endogenous carcinogens. Emphasis will be placed on mixed function oxidase systems of microsomes derived from liver and small and large intestines. Investigate the role of bile acids and sterols in the carcinogenesis of the colon with emphasis on the interaction between chemicals and microflora. Investigate the role of intestinal epithelial cells in the metabolism of luminal substrates as well as intestinal DNA damage and repair as a marker to identify intestinal carcinogens and modifiers.

4. Endocrine Carcinogensis

Contractor's Project Manager: Dr. Douglas H. Janss

Objectives: To understand DMBA carcinogenesis and steroid mechanism of action in the rat mammary gland using in vivo and in vitro systems, to elucidate the key events of these processes and to utilize human breast tissue in vitro to correlate animal data with the human. Development of an in vitro system for the study of mammary epithelial cell replication, differentiation and transformation and to use the model to investigate the mechanism of mammary carcinogenesis. To assess the importance of steroid hormones in the maintenance of cultures of rat and human mammary epithelial cells and in the induction of mammary cancer in rats by dimethylbenz-[a]anthracene.

Major Findings: Epithelial elements have been isolated from the mammary fat pad of Sprague-Dawley female rats at the age of maximum sensitivity to DMBA-induced tumorigenesis. They have been cultured in vitro and characterized for their requirements for specific hormones at physiological levels to insure normal growth and morphology. A method for the routine isolation and growth of large numbers of normal breast epithelial cells have been developed using collagenase dissociation of human breast tissue which results in a cell mixture of enriched epithelial elements. These culture grow and maintain normal morphology under the influence of physiological levels of specific hormones. The hydrocarbon metabolizing enzyme, aryl hydrocarbon hydroxylase, was compared in mammary epithelial cells and fibroblasts and studies suggested that constitutive and induced AHH activity in primary cultures of rat mammary epithelial cells and fibroblasts were controlled by separate regulatory mechanisms.

Metabolism of dimethylbenz[a]anthracene has been examined in mammary gland and epithelial cells from rat and humans and some metabolites in rat and human mammary tissue identified. Metabolism in a human breast tumor cell line was different.

Comparison of binding of DMBA to DNA of liver and mammary gland in vivo showed that binding to mammary DNA was higher than in liver at all ages, but was maximal at the optimal age for mammary tumor induction by DMBA.

Significance to Biomedical Research and the Program of the Institute: A comprehensive model system for the study of human breast cancer has been developed which includes an in vivo DMBA rat mammary epithelial cell element and an in vitro normal human breast epithelial cell system. Since the induction of tumors in the rat system is dependent upon the same series of factors (age, genetics and endocrine status) which modulates human breast cancer indidence, this comprehensive model can be used to elucidate the mechanisms whereby these factors initiate human breast cancer.

Proposed Course: To examine the influence of age, genetics and endocrine status on the progression of events which lead to the loss of the ability

of an immature cell to respond to hormonal stimuli which signal for ultimate differentiation and specific function and, thus, allow the immature cell to retain its ability to divide and form a transformed cell mass. This system will also be used to examine the question of whether hormones (naturally occuring or synthetic products) might induce neoplasia through mechanisms which are typical of carcinogen or hormonal action. To investigate further the significance of the interaction of DMBA metabolites with steroid receptors to the process of carcinogenesis.

5. Molecular Aspects of Chemical Carcinogenesis

Contractor's Project Manager: Dr. Anthony Dipple

Objectives: The specific objectives of this section are to define aspects of the process of tumor initiation which may be common to a wide range of chemical carcinogens so that better means of detection of carcinogens and of prevention and treatment of cancer in man can be developed.

Major Findings: The mechanism through which the most potent hydrocarbon carcinogen, 7,12-dimethylbenz[a]anthracene (DMBA), is activated by metabolism has been clarified. These findings support a generalization due to Jerina and Daly which suggests hydrocarbons in general are activated through bay region diol-epoxides. Thus, careful studies of the photosensitivity of DMBA-DNA adducts revealed that metabolic activation of this carcinogen either leaves the whole aromatic system intact or results in the saturation of the 1,2-and 3,4-double bonds. Examination of the fluorescence properties of model compounds and DMBA-DNA adducts established that, in fact, the 1,2-and 3,4-double bonds are saturated. The inhibition of binding of DMBA to DNA in intact mammalian cells by an inhibitor of epoxide hydrase, TCPO, confirmed that a diol mediated the binding. Therefore, it has been established that a diol-epoxide generated in the 1,2,3,4-ring of DMBA (not in the 8,9,10,11-ring as had been suggested by others) is involved in its binding to DNA and probably also in its carcinogenic action. Additionally, it has been shown that in microsomal systems, analogous to those used in conjunction with bacterial mutagenesis assays, a reactive K-region oxide, not a 1,2,3,4-ring diol-epoxide, is generated. Thus, mutagenicity in this case is not based on the same properties as carcinogenicity and different activation systems are required to properly use mutagenesis as an indicator of carcinogenic activity for the hydrocarbons.

Quantitative aspects of excision of chemical damage in replicating mammalian cells have been clearly documented. These show that, even under conditions leading to 100% survival, DNA replication occurs on an extensively damaged template. Major differences in the repair response to chemicals of different carcinogenic potency have not been detected. It has been shown that, in a range of different mammalian cells, excision is somewhat selective and the selectivity observed is of the same order as that demonstrated by an N-glycosidase activity in vitro. A range of human

cells deficient in repair with respect to uv-damage are similarly deficient with respect to chemical damage and their survivals are clearly related to their repair capacities.

The most exciting developments in these studies are currently arising through the chemical studies of alkylation at various sites in nucleic acids. It is becoming clear that reaction at ring nitrogen atoms arises through kinetically controlled processes in which any selectivity would reside in the nucleophile itself. However, reaction at exocyclic sites required an ionic intermediate under thermodynamic control in which case selectivity would reside in the reactive intermediate itself. Since most carcinogens given rise to some exocyclic substitution, it seems that the common factor amongst chemical carcinogens is their ability to generate an ionic intermediate whose selectivity permits it to modify some critical cellular receptor.

Significance to Biomedical Research and the Program of the Institute: One of the goals of the National Cancer Institute is to prevent cancer in man through the identification and detection of environmental carcinogens. Basic research aimed at identifying common features amongst chemical carinogens or in their mechanism of action should provide a rational basis for the improvement of existing procedures and for the development of new procedures for the identification and detection of chemical carcinogens.

Proposed Course: Research will be conducted to pursue and confirm the observations described above. This will include: (1) Attempts to define better metabolic activation systems for the mutagenesis assays of hydrocarbon carcinogens. (2) More precise definitions of the relationship between chemical reactivity and carcinogenic potency. (3) Investigation of the role of chemical reactivity in determining selectivity of reaction with various subcellular, chromatin, or DNA fractions and the relationship of this selectivity to carcinogenic activity. (4) Better definition of the role of excision repair processes in permitting or preventing tumor initiation. (5) Investigate relationship between metabolic activation of DMBA for DNA binding and tumorigenesis by DMBA in mouse skin.

6. Cofactors in Chemical Carcinogenesis

Contractor's Project Manager: Dr. Winifred G. Palmer

Objectives: To determine the role macrophages play in tumorigenesis by metabolizing polyaromatic hydrocarbons (PAH) to reactive derivatives which are released and taken up by susceptible target cells.

Major Findings: Macrophages convert DMBA into derivatives which are generally less polar than those produced by other cells and tissues. Unlike intact tracheal and lung tissue, macrophages tended to release most of the derivatives that they produced into the surrounding medium. These derivatives were readily accumulated intracellularly by lung and tracheal tissue when they were co-cultivated with macrophages in the presence of DMBA.

Macrophages continued to actively metabolize DMBA at doses of DMBA which reduced the AHH activity in respiratory tissues. The proportion of metabolites produced by macrophages changed as the DMBA dose was increased. Similar alterations in the proportions of metabolites were produced by incubating macrophages with the AHH inhibitor, 7,8-benzoflavone. These data suggest that there may be two different aryl hydrocarbon hydroxylases present in macrophages which are responsible for the conversion of DMBA to different derivatives.

Significance to Biomedical Research and the Program of the Institute: The potential role of macrophage metabolism of carcinogenic substances in pulmonary carcinogenesis is unknown. This project has been directed toward this objective.

Proposed Course: The current set of experiments has come to its conclusion; there are no plans to continue these studies at this time.

7. Mechanisms of Nitrosamine Carcinogenesis

Contractor's Project Manager: Dr. William Lijinsky

Objectives: To investigate the formation of carcinogenic nitrosamines in the environment and in vivo, using chemical and biological systems. To investigate the relationship between chemical structure of nitrosamines and their carcinogenic activity, differences in potency and species and organ specificity among nitrosamines of similar structure, and to elucidate biochemical pathways that relate to carcinogenesis by nitrosamines.

Major Findings: Approximately 1200 rats have been placed on test to investigate (a) formation of carcinogenic nitrosamines in vivo from amines and nitrite, (b) structure activity relations among cyclic and acyclic N-nitroso compounds. In several of the latter studies almost all of some groups are dead and histopathology has been performed on them.

Tests of 5 nitrosamines in Guinea pigs (140 total) in progress have shown that all are carcinogenic, but induce tumors in different organs from those susceptible to those compounds in the rat.

A variety of N-nitroso compounds has been synthesized having several structural characteristics. These are being evaluated by testing their carcinogenicity and mutagenicity which will support or contradict present hypotheses of the mechanism of carcinogenic action of N-nitroso compounds. Among them are several deuterium labeled nitrosomethylethylamines and nitrosoalkylcarbamates, as well as deuterated cyclic nitrosamines.

Dose response studies with some N-nitroso compounds are in progress to establish baselines for extrapolation to low doses. Especially important is whether there are differences in shape and slope of the dose response curves from one carcinogen to another.

The mechanisms of nitrosation of tertiary amines and of transnitrosation by aliphatic and alicyclic nitrosamines have been investigated in an ongoing program. Both mechanisms are complex and the reaction of tertiary amines with nitrous acid leads to formation of an array of products, of which only some have been identified as yet.

The metabolism of nitrosopyrrolidine, nitrosohexamethyleneimine, nitrosoheptamethyleneimine and 2,6-dimethylnitrosomorpholine have been examined in both target and non-target organs of the rat and, in the case of dimethylnitrosomorpholine, in the Syrian hamster. Several metabolites have been isolated but most of them await identification. Differences in metabolism have been seen between target and non-target organs, both in vivo and in vitro, which might be correlated with the differences in carcinogenic action of the compounds.

Significance to Biomedical Research and the Program of the Institute: Nitrosamines are a group of broadly acting potent carcinogens which might play a considerable role in carcinogenesis in man. Investigation of their formation in the environment and in vivo can indicate how significant that role is. Studies of the biochemistry and chemistry of nitrosamines relating to their carcinogenic action can help in understanding carcinogenesis in general.

Proposed Course: The current activities will continue in examining the mechanism of formation of nitrosamines from their precursors, the relation between chemical structure and the carcinogenic activity of nitrosamines, and biological studies in animals, such as dose-response and organ specificity, that relate to risk extrapolation. Biochemical studies of the mechanism of action of selected carcinogenic nitrosamines will be conducted.

8. Microbial Mutagenesis

Contractor's Project Manager: Mr. A. W. Andrews

Objectives: Efficient, reproducible, and reliably performed screening assays are provided for any and all compounds submitted for testing in a bacterial mutagenesis detection system. Different chemicals are handled in a variety of ways to determine whether or not they are capable of mutating any of eight strains of Salmonella bacteria. Various mammalian organs and enzyme systems as well as different solvents and exposure techniques are used.

Major Findings: The laboratory procedures for the large scale screening of samples using the Salmonella/mammalian-microsome mutagenicity test have been organized so that several different applications of this assay are possible.

Each compound is tested using the 5 standard Salmonella tester strains (TA 1535, 1537, 1538, 98 and 100) and an Aroclor 1254-stimulated or

phenobarbital-stimulated rat liver homogenate (S_9 mix) in the plate assay. If mutagenesis is observed a 10 point dose-response curve covering a thousand-fold range is done using the strain or strains which show optimum mutagenesis. If the plate assay gives negative results, the liquid assay, wherein the bacteria, test compound and S_9 mix are combined for varying lengths of time at different temperatures is often the best method for demonstrating mutagenesis.

A collection of enzyme preparations from organs other than the liver has been assembled, including brain, esophagus, lung, intestine, spleen, kidney and testes. Many chemicals that are currently non-mutagenic may be converted to mutagenic metabolites if exposed to different enzyme systems.

During the last year 424 samples were tested including azo dyes, cell extracts, cholesterol derivatives, intestinal mucosal extracts, bile acids, fecal extracts and their metabolites, polynuclear hydrocarbons N-nitroso compounds, potential cancer therapeutic agents, suspect compounds for bioassay and several commonly used drugs. Comparison of mutagenic and carcinogenic potency has been made among many nitrosamines and polynuclear hydrocarbons.

Significance to Biomedical Research and the Program of the Institute: The development of the *Salmonella*/mutagenesis test as a predictive screen for potential carcinogens is important as part of a battery of short term tests to precede long term bioassays in animals. Validation of the test against a series of known carcinogens is essential, and application of it to many substances proposed for bioassay, at FCRC and elsewhere, is most useful.

Proposed Course: Comparison of the test with groups of known carcinogens will continue. Development of activation systems from organs other than the liver will increase the usefulness of the test and may avoid the false negatives. The Project will continue to be a resource for mutagenesis testing of a wide variety of materials of interest to the research programs at FCRC.

9. Chemistry of Carcinogens

Contractor's Project Manager: Dr. Christopher J. Michejda

Objectives: Several classes of chemicals have been identified as carcinogens in animals, and by extension, in man. At the present time, the mechanism of cancer induction by chemicals is unknown. It is the objective of this research to learn about how and why some of these chemical classes are carcinogenic. The chemicals include nitrosamines, triazenes, tetrazenes, derivatives of hydroxylamines and hydrazines. The fundamental chemical transformations of these substances, which may have bearing on their carcinogenicity, will be examined. The mechanisms of enzymatic reactions, which transform these compounds into the ultimate carcinogens, will be studied.

Major Findings: The steady-state kinetics for the formation of formaldehyde from dimethylnitrosamine (DMN) and DMN-d_6 as well as phenylmethylnitrosamine (PMN) and PMN-d_3 have been determined. This allowed the calculation of the kinetic isotope effects for both of the nitrosamines (K_H/K_D (DMN) = 1.8; K_H/K_D (PMN) = 5.4), which indicated that the rate determining step in the enzymatic oxidation of these nitrosamines is the breakage of the C-H bond. These kinetic data also suggest (although do not prove) that the result of the bond breakage is a carbanion, which is then oxidized rapidly.

The formation of formaldehyde from PMN requires that the other fragment be the phenyl diazonium ion. This was proved by trapping experiments. The amount of the diazonium ion correlated precisely with the amount of formaldehyde. Analogous experiments with DMN turned out to be very important. In collaboration with Dr. Larry Keefer of NCI, some unsymmetrically labeled DMN-d_3 (label in only one methyl group) was obtained. Kinetic analysis revealed that the methyl group anti to the nitroso oxygen undergoes enzymatic oxidation. Preussmann reported the trapping of the methyl group from DMN and other nitrosamines using the nucleophile, 2,4-dichlorothiophenol. The present experiments revealed that only about 1% of Preussmann's trapped methyl came from the nitrosamine. These experiments demand that there must be considerable methylating activity in the microsomes. This renders suspect all the methylation studies which used microsomes to activate the nitrosamines. This result may also have a profound effect on mutagenicity data obtained using microsomal activation.

The post-microsomal fraction of a rat liver homogenate contains a soluble enzyme which is active in nitrosamine hydroxylation. A partial purification of this enzyme has been carried out and some kinetic parameters have been determined. Carbon monoxide inhibition studies have revealed that this is not a cytochrome P-450 enzyme.

Capitalizing on earlier finding of anchimeric assistance by the N-nitroso group, a number of β-tosyloxy and γ-tosyloxynitrosamines have been prepared. A study of the solvolysis of these materials revealed that β-tosyloxynitrosamines are extremely reactive (the tosyloxy group is simply an excellent leaving group). This makes them into potential direct acting alkylating agents. It has been found (in collaboration with Mr. Wes Andrews of FCRC) that methyl-(β-tosyloxy-ethyl)-nitrosamine is a powerful mutagen without activation. The closely related γ-tosyloxy-propyl derivative is not. It was also found that the aforementioned β-tosyloxynitrosamine is an excellent methylating agent for guanine.

A number of vinyl nitrosamines have been prepared by a vastly improved procedure, which will make this interesting class of compounds available for study. A new class of nitrosamines , α-epoxynitrosamines have been prepared. These offer many exciting possibilities, among them being the possible isolation of the putative α-hydroxynitrosamine.

Significance to Biomedical Research and the Program of the Institute: The understanding of the fundamental chemistry of nitrosamines is a

sine qua non in the understanding of the metabolism of these substances, which leads to the carcinogenic response. The understanding of the mechanism of the enzymatic transformation of nitrosamines will provide a basis for molecular level understanding of carcinogenicity. The discovery of the alkylating ability of β-hydroxynitrosamines provides a possible new route for activation of nitrosamines.

Proposed Course: Research will be conducted on further aspects of the problems discussed above. Contrator will also examine triazenes and tetrazenes, as well as some free radical reactions of nitrosamines.

10. Cellular Aspects of Chemical Carcinogenesis

Contractor's Project Manager: Dr. P. Thomas Iype

Objectives: The primary objective of this Section is to study the mechanism of the carcinogenic process paying special attention to the role of target cells and tissue organization. The information obtained will be helpful in the development of effective diagnostic, preventive and therapeutic methods for cancer. The immediate goal is to increase the sensitivity of transformation assays for epithelial cells.

Major Findings: This Section was started in July 1977. Initially, considerable time was devoted to the organization of the laboratory. The conversion of an administrative area into an effective, comprehenisve biological laboratory was planned. This laboratory is now nearing completion. A staff of two technicians and a Scientist has been hired.

Using the well-characterized rat liver epithelial cells brought from a previous laboratory, a number of experiments have been done in a temporary laboratory. After standardizing culture materials, experimental conditions which would favor the enrichment of transformed cell population in a mixed culture were investigated. It was established that 7,12-dimethylbenz-(a)anthracene (DMBA) selectively kills normal hepatic cells with minimal effects on hepatoma cells. Since cytotoxicity is generally ascribed to the metabolic capacity of cultured cells, a comparison was made of the metabolism of DMBA by normal and malignant rat liver cells. It was found that both cell types could metabolize [^{3}H]-DMBA and that the metabolites in both cases were qualitatively similar. The binding of [^{3}H]-DMBA to cellular DNA also occurred in both cases.

Both dimethyl sulfoxide and glycerol at certain concentrations are selectively toxic to normal liver cells. Preliminary experiments on the cytotoxic effects of benzo(a)pyrene, 3-methylcholanthrene, -amanitin, 2-acetylaminofluorene and aflatoxins towards normal liver cells were performed. Adult rat serum has a differential cytostatic action towards normal rat liver cells, and adult rat liver chalone fractions have been prepared for use as a possible selective agent.

Viable mouse prostate cells could be isolated by treatment with the enzyme "dispase", and special culture media have been prepared for their propagation.

Significance to Biomedical Research of the Program at the Institute: The majority of human tumors are epithelial in origin and it is important to investigate the mechanism of carcinogenesis in epithelial cells. Improvements in the in vitro assays for transformation, and the development of selective factors to enrich the transformed cell population will increase the sensitivity of screening systems in general and also make possible the elucidation of the chronological events which occur during the carcinogenic process. The information gained could be used to devise means which would interfere with these early carcinogenic changes, thereby preventing the tumor from developing.

Proposed Course: Research in the immediate future will be directed towards the achievement of two short-term objectives, i.e., to establish (1) objective criteria relevant to epithelial cell transformation and (2) experimental conditions which select transformed epithelial cells to increase the sensitivity of the assay using methods involving the use of different cytotoxic and cytostatic agents. Reported biochemical differences between normal and transformed cells will be used to create selective pressure to prevent normal epithelial cell division.

NEBRASKA, UNIVERSITY OF (EPPLEY INSTITUTE FOR RESEARCH ON CANCER) (NO1-CP-33278)

Title: A Resource for Carcinogenesis Bioassays and Related Research

Contractor's Project Director: Dr. David B. Clayson

Project Officers (NCI): Dr. Richard Griesemer
Dr. David Longfellow
Dr. Carl Smith

Objectives: The bioassay of suspected chemical carcinogens, definition of the biological and chemical mechanisms of the carcinogenesis process and the analysis of trace levels of environmental carcinogens are three principal research aims under this contract.

Major Findings: A. Pancreatic Carcinogenesis--Selective induction of pancreatic cancer in the Syrian hamster: Single injections to Syrian golden hamsters of N-nitrosobis(2-oxopropyl)amine in doses corresponding to 1/2.5, 1/5, 1/10, 1/20 and 1/40 of the LD50 resulted in induction of pancreatic neoplasia in incidences of 67, 53, 53, 27 and 7%, respectively. In most animals, the pancreatic tumors were the only induced tumors. The tumor latency was 30 ± 9 weeks at the highest and 44 ± 12 weeks at the lowest dose levels. Ductular cells of the pancreas seem the most responsive to this carcinogen.

The effect of cholecystoduodenostomy and choledochostomy in pancreatic carcinogenesis: N-nitrosobis(2-oxopropyl)amine was administered to Syrian golden hamsters after cholecystoduodenostomy and choledochostomy to investigate a possible bile reflux mechanism as a factor in pancreatic carcinogenesis. The induced lesions were similar in morphology, multiplicity, and distribution to those in animals without surgery. The findings hence contra-indicate the importance of biliary reflux in pancreatic tumor induction.

Evidence of ductular cell origin of pancreatic cancer and its multiplicity in humans comparable to that in the experimental model: Systematic histologic examination of three patients, who died of pancreatic cancer and were autopsied by the pathology department in the University of Nebraska revealed that in all 3 cases tumors were of multiple origin. In one case, more than 200 preneoplastic and neoplastic lesions could be identified in different pancreatic segments. Another similarity between the hamster model and human cases were tumors which seemed to arise from ductules rather than from ducts. This observation, which further substantiates the significant role of hamsters in pancreatic carcinogenesis studies, opens an area for investigating the etiologic factors involved in this cancer site.

Induction in rats of colorectal tumors which closely resemble the equivalent lesion in man: Weekly s.c. injection of N-nitrosobis(2-oxopropyl)-amine, a known potent pancreatic carcinogen in hamsters, induced colorectal cancer in MRC rats in 67% of all males and 33% of all females that survived beyond 43 and 68 weeks, respectively. Tumors were concentrated in specific segments of the large bowel and not found in the small intestine. Although the distribution of cancer of the cecum and that of the ascending and descending colon was similar in both sexes, rectal cancer predominated significantly in males. These data and the tumor morphology indicates the present model more closely resembles the corresponding human disease, than do those in previous relevant experimental studies. Pancreatic tumors were not induced by this compound in rats.

The hamster pancreatic carcinogens N-nitrosobis(2-hydroxy)propylamine (BHP) and N-nitrosobis(oxopropyl)amine (BOP) were synthesized with ^{14}C-label for further metabolic studies. Both compounds were extensively metabolized to $^{14}CO_2$. The urine of hamsters administered each compound contained several as yet unidentified nitrosamine metabolities, as well as N-nitroso(2-hydroxypropyl)(2-oxopropyl)-amine (HPOP). Binding of ^{14}C to DNA and protein of various tissues was determined after BHP administration and "binding" to hamster pancreas protein was the highest. There were quantitative differences in the metabolism of BHP and BOP in the rat, a species in which these compounds are not pancreatic carcinogens, as opposed to the hamster. The latter situation may yield clues to the organotropic effects of these compounds.

Another study in the pancreatic cancer area concerns N-nitroso-2,6-dimethylmorpholine (NDMM), a mixture of two geometric isomers. Subcutaneous administration of the mixture has induced pancreatic tumors in hamsters.

The cis isomer is metabolized in vivo more readily than the trans isomer by β-oxidation to HPOP, which was proposed to be the mechanism of pancreatic carcinogenesis. However HPOP is less mutagenic in the Ames assay than is NDMM, and the two isomers are approximately equally active as mutagens, indicating that the mutagenic activation using hamster liver preparation is not by β-oxidation.

B. Hydrazine Carcinogenesis--Investigation continued in the area of hydrazines. Among the past year's findings were that phenylethylhydrazine (Nardil: Phenelzine), used clinically as an antidepressant, induced blood vessel and lung tumors in mice.

Pyridoxine hydrochloride (vitamin B_6) was shown to inhibit the toxicity of four substituted hydrazines (methyl-, ethyl- and butylhydrazine and β-N[α-L(+)-glutamyl]-4-hydroxymethylhydrazine). Studies are currently underway to determine whether pyridoxine also inhibits the carcinogenicity of these substances.

The edible false morel (Gyromitra esculenta) (a mushroom) contains appreciable quantities of N-methyl-N-formylhydrazine. This hydrazine derivative is extremely hepatotoxic in mice, and when fed at doses compatible with survival (0.068% in the diet), led to tumors of lungs, liver, blood vessels, bile duct and gall bladder within the unusually short period of 30 weeks.

Another mushroom, the commonly eaten cultivated Agaricus bisporus contains 4-hydroxymethylphenylhydrazine. Feeding the acetyl derivative of this relatively unstable substance led to tumors of lungs and blood vessels in mice.

Succinic acid 2,2-dimethylhydrazide is a plant growth regulant used in the U.S.A. and chemical carcinogen. When administered orally to mice, it produced tumors of blood vessels, lungs and kidneys.

Agaritine (beta-N-(gamma-glutamyl)-4-Hydroxymethylphenylhydrazine) is a constituent of the common edible mushroom Agaricus bisporus. An enzyme found in the mushroom catalyzes the hydrolysis of agaritine to glutamic acid and 4-hydroxymethylphenylhydrazine. The hydrazine component, which is unstable, can be trapped as a hydrazone in the presence of sodium glyoxalate. A relatively non-specific gamma-glutamyl transpeptidase from hog kidneys also catalyzes this hydrolysis, slowly but quantitatively. This shows that conditions exist in the mammalian system for the breakdown of agaritine to a compound that is likely to be carcinogenic. It has also been found that agaritine, in the absence of this enzyme, is still partially (ca. 20-30%) converted to 4-hydroxymethylphenylhydrazine in acidic, basic, or heated aqueous solutions.

C. Mechanisms--Highlights of selected research projects on the mechanism of carcinogenesis are as follows:

In the area of polycyclic aromatic hydrocarbons, the following results were shown: (a) Approach to assess the nature of proximate carcinogens for alternant aromatic hydrocarbons: Different activation mechanisms are responsible for the carcinogenic activity of the various polycyclic aromatic hydrocarbons (PAH). The carcinogenicity of alternant PAH can best be understood in terms of three major mechanisms which lead to three proximate carcinogens: arene oxides, radical cations and hydroxy derivatives at the benzylic carbon atom of the alkyl-substituted PAH.

A correlation exists between ease of formation of radical cations and carcinogenic activity, if one excludes hydrocarbons in which arene oxides and hydroxymethyl derivatives are the important proximate carcinogens. This has been done by determining ionization potentials (IP's), which are a measure of the hydrocarbon's ability to form a radical cation. IP's of ca. 80 PAH were determined or re-determined from absorption maxima of the charge-transfer complex with chloranil. This study demonstrated that the carcinogicity of alternant PAH can best be understood in terms of three separate mechanisms applicable to three different categories of PAH: (1) Unsubstituted PAH with IP's above ca. 7.35 eV, in which arene oxides are postulated to be the ultimate carcinogens (e.g., dibenz[a,h]anthracene, dibenz[a,c]anthracene, chrysene, benz[a]anthracene and benzo[c]phenanthrene). (2) Alkyl-substituted PAH with IP's above ca. 7.35 eV in which hydroxylation at the benzylic carbon atom is the major pathway to carcinogenesis (monomethylbenz[a]anthracenes, monomethylbenzo[c]phenanthrenes, and 5-methylchrysene). (3) Substituted and unsubstituted PAH with IP's below ca. 7.35 eV, in which radical cations are the ultimate carcinogens (e.g., 7,12-dimethylbenz[a]anthracene, 3-methylcholanthrene, benzo[a]pyrene and the dibenzo[a]pyrenes).

(b) Binding of benzo[a]pyrene in various biological systems: Studies of the mechanism of binding of polycyclic aromatic hydrocarbons to nucleic acids have been conducted in several biological systems: in vitro with isolated rat liver nuclei and microsomes and in vivo on mouse skin. Syntheses of the following specifically tritiated benzo[a]pyrenes were developed: 6-, 1,3-, 1,3,6-, 7-, and 6,7-. Loss of tritium upon binding of B[a]P to DNA or RNA in mouse skin or rat liver nuclei occurs predominantly at the 6 position. These results support one-electron oxidation as the principal mechanism of activation of this carcinogen.

(c) Binding of aromatic hydrocarbons catalyzed by horseradish peroxidase: A biological system has been devised in which activation of a polycyclic aromatic hydrocarbon by a purified enzyme can be studied. Horseradish peroxidase catalyzes the binding of benzo[a]pyrene and 3-methylcholanthrene to DNA. The mechanism of activation appears to be one-electron oxidation. In addition to providing a purified model system for studies of hydrocarbon activation, peroxidase may be the activating enzyme in mammary gland carcinogenesis by hydrocarbons.

(d) Differences in nuclear and microsomal cytochrome P-450: Cytochrome P-450 in rat liver nuclei has been found to differ from microsomal

cytochrome P-450 in its CO- and ethyl isocyanide-binding spectra. Differences were seen in uninduced rats and those treated with 3-metylcholanthrene or phenobarbital. These differences have been correlated with other properties which distinguish nuclear monooxygenase enzymes from microsomal.

(e) Synthesis of sulfate esters: A general synthesis of sulfate esters of hydroxyalkyl-substituted PAH has been developed as part of a program to evaluate the role of these potential metabolites in carcinogenic activation of alkyl-substituted PAH. The synthesis has been applied to esters of 6-hydroxymethylbenzo[a]pyrene, 7- and 12-hydroxymethylbenz[a]anthracene, 5-hydroxymethylchrysene and 7-(β-hydroxyethyl)benz[a]anthracene. Sulfate esters of the benzylic alcohols are strongly mutagenic toward strain TA 98 of Salmonella Typhimurium, in contrast to the parent alcohols and the nonbenzylic esters, which are virtually inactive. Tests for carcinogenicity and metabolic formation of this class of conjugates have been initiated.

(f) Carcinogenicity and metabolism of 7-methylbenz[a]anthracene derivatives: The relative carcinogenicity of 7-methylbenz[a]anthracene (BA-7-CH) and some of its derivatives was quantitatively compared in a dose-response experiment by repeated application on mouse skin. The stronger carcinogenicity of 7-acetoxymethylbenz[a]anthracene relative to that of hydroxymethylbenz[a]anthracene ($BA\text{-}7\text{-}CH_2OH$) suggests that the activity of the latter compound may be related to the formation of an ester, which is able to act as an ultimate reactive alkylating compound. The similar or weaker carcinogenic activity of benz[a]anthracene-7-carboxaldehyde (BA-7-CHO) relative to that of $BA\text{-}7\text{-}CH_2OH$ and the metabolic reduction of BA-7-CHO to BA-7-CH OH in mouse skin suggest the latter compound might be its proximate carcinogenic metabolite. The stronger carcinogenicity of $BA\text{-}7\text{-}CH_2OH$ relative to that of $BA\text{-}7\text{-}CH_3$ and the formation of $BA\text{-}7\text{-}CH_2OH$ as the major metabolite of $BA\text{-}7\text{-}CH_3$ in mouse skin suggest that $BA\text{-}7\text{-}CH_2OH$ is likely to be an important proximate carcinogen of $BA\text{-}7\text{-}CH_3$.

(g) Carcinogenicity and metabolism of 6-methylbenzo[a]pyrene derivatives: In a dose-response experiment, the relative carcinogenicity of 6-methylbenzo[a]pyrene ($BP\text{-}6\text{-}CH_3$), 6-hydroxymethylbenzo[a]pyrene ($BP\text{-}6\text{-}CH_2OH$), benzo[a]pyrene-6-carboxaldehyde (BP-6-CHO) and the newly synthesized 6-hydroxymethylbenzo[a]pyrene sulfate ester ($BP\text{-}6\text{-}CH_2OSO_3Na$) was determined by repeated application on mouse skin. The weaker carcinogenicity of BP-6-CHO relative to that of $BP\text{-}6\text{-}CH_2OH$ and the efficient metabolic reduction of BP-6-CHO to $BP\text{-}6\text{-}CH_2OH$ in mouse skin suggest the latter compound might be its proximate carcinogenic metabolite. The lesser carcinogenicity of $BP\text{-}6\text{-}CH_2OH$, relative to that of $BP\text{-}6\text{-}CH_3$, indicates that the former cannot function as the major proximate carcinogenic metabolite for $BP\text{-}6\text{-}CH_3$, although it is not excluded that this oxidation pathway might contribute to some extent to the $BP\text{-}6\text{-}CH_3$ activity. The stronger carcinogenicity of $BP\text{-}6\text{-}CH_2OSO_3Na$ relative to that of $BP\text{-}6\text{-}CH_2OH$ suggests that the activity of the latter compound may be related to the formation of an ester, which is able to act as an ultimate reactive alkylating compound.

Highlights of the nitrosamine program were as follows:

(h) The high incidence of esophageal cancer in the Transkei, South Africa, could be partly due to the consumption of a green vegetable *Bidens pilosa*. A system was developed for measuring [^{3}H]thymidine incorporation (TI) in rat esophageal epithelium to detect esophageal carcinogens. (Complete carcinogens cause an acute *inhibition* of TI.) The effect on TI of BDP added to the diet as dried leaves and fed for one week to rats has now been studied. A significant *enhancement* of TI, up to 2.3 times the control level was obtained. The effect was due to an alcohol-soluble component of *B. pilosa* and occurred both with *B. pilosa* from the Transkei and with that grown in the U.S.A. An enhancement of TI usually precedes hyperplasia, which in turn is often associated with tumor promotion. Hence the results suggest *B. pilosa* consumption may be significant factor in the high incidence of esophageal cancer in South Africa.

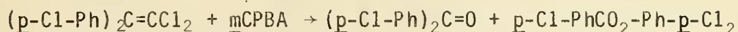
(i) The kinetics of nitrosamide formation in organic solvents were studied. The nitrosating agents were a dried nitrous acid extract and dinitrogen tetroxide. In methylene chloride solution, nitrosation of common ureas and carbamates, e.g. ethylurea, proceeded so rapidly that the reaction was almost complete in 5 seconds at 4°C. For *N*-butylacetamide nitrosation under one set of conditions, the rate constant was 31,000 times that for the same reaction in water at pH 2. The very rapid reaction in organic solvents may be the mechanism for formation of *N*-nitroso compounds under certain industrial situations (e.g., in agricultural chemicals) and in fried bacon.

(j) Since nitrosamide formation in vivo may be involved in the etiology of gastric cancer, contractor determined the amount of methylnitrosourea (MNU) in the stomach of rats 3 hours after they were fed methylurea (MU) in the diet (100 mg/kg), together with sodium nitrite. A radioactive method was used for analysis. When MU and NaNO (2g/kg) were mixed in a semisynthetic diet, the MNU yield was 1.4% of the MU. The MNU was still detected (yield, 0.2%) when $NaNO_2$ was lowered to 0.5 g/kg. When $NaNO_2$ (2 g/l) was added to to the drinking water, the MNU yield was 0.53% and under these conditions MNU was still detected (yield, 0.08%) at an $NaNO_2$ concentration of 0.5 g/l. Lower concentrations of $NaNO_2$ have not been shown to yield MNU. These results are 6-13 times higher than comparable results when a commercial rat diet was used, probably because the semi-synthetic diet buffered the gastric acid less efficiently. This suggests that diet is a determinant of in vivo nitrosation, through an effect on gastric pH.

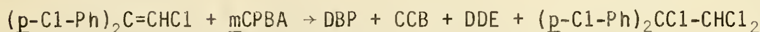
Additional highlights include:

(k) The peracid epoxidation of two metabolities of DDT, 1, 1-dichloro-2, 2-bis(*p*-chlorophenyl)ethene (DDE) and 1-chloro-2, 2-bis(*p*-chlorophenyl)-ethene (DDMU) was investigated. The oxidation of DDE using *m*-chloroperbenzoic acid yielded 4, 4'-dichlorobenzophenone (DBP) and its Baeyer-Villiger oxidation product 4'-chlorophenyl-4-chlorobenzoate (CCB). The oxidation of DDMU afforded DBP, CCB, DDE and 1, 1, 2-trichloro-2, 2-bis(*p*-chlorophenyl)ethene (DTE). The formation of DDE and DTE resulted from a

chlorination of DDMU, which is inhibited in the presence of anisole. Anisole trapped the chlorinating agent formed during the oxidation to form p-chloroanisole and no DDE or DTE was observed.



DDE DBP CCB



DDMU DTE

(l) Ten thousand xg supernatant (S-9) from various organs and species of control or phenobarbital- and aroclor-induced rodents have failed to activate several different hydrazine derivatives. Mutagenic activity in the absence of S-9 is found with high levels of tolyl and phenylhydrazines. 2, 4-Dinitrophenylhydrazine is also mutagenic in the absence of S-9, but metabolism at the nitro group by the bacteria is considered the source of mutagenic potential with this compound.

(m) Enzymatic methods are being used to isolate epithelium from underlying connective tissue and muscle in rat, mouse and bovine urinary bladder. These isolated cells are being used for short-term metabolism-related mutagenesis studies using V-79 cells and for long-term culture of urinary bladder epithelium for the establishment of cloned epithelial cell lines. A number of known carcinogens have been screened on the V-79 cells alone with little or no significant mutation rates in most cases, indicating that this is a valid test system for metabolism-related mutagenesis studies. Several known bladder carcinogens are currently being tested with this system in the presence of bladder epithelial cells to study the role of bladder metabolism in the formation of activated carcinogens.

Significance to Biomedical Research and the Program of the Institute: The Eppley Institute has provided over the years a considerable output of major research results in chemical carcinogenesis and represents a prime national resource in which chemical, pathological and biological aspects of carcinogenesis are studied in an integrated program.

Proposed Course: This contract will continue to support the study, improvement and conduct of bioassay techniques and research into chemical mechanisms of the carcinogenesis process, as well as analytical studies of environmental carcinogenesis. Work will continue as outlined above.

Date Contract Initiated: March 19, 1968

Current Annual Level: $2,223,359*

* Carcinogenesis Research Program funds only. For information on funding levels for the Carcinogenesis Testing Program, see the 1978 Annual Report prepared by that Program Area.

NEW HAMPSHIRE, UNIVERSITY OF (NO1-CP-55675)

Title: Preparation of Various N-Nitroso Compounds

Contractor's Project Director: Dr. Robert E. Lyle

Project Officer (NCI): Dr. Larry K. Keefer

Objectives: (1) To develop improved synthetic methods for carbon labeled heterocyclic nitrosamines of potential interest in carcinogenesis research. Such compounds might include N-nitrosopiperidine, N-nitrosopyrrolidine and others at the direction of the project officer, (2) To prepare pure samples of a number of unlabeled N-nitroso compounds, to be identified by agreement with the project officer, and deliver them safely to the Carcinogenesis Standard Reference Compound Bank, and (3) To investigate other synthetic approaches to radio-labeled N-nitroso compounds as may be mutually agreeable to the Principal Investigator and the Project Officer.

Major Findings: The properties of nitrosamines provide a convenient route to substituted derivatives. Treatment of nitrosamines with base gives an anion which can be converted to a variety of new nitrosamine derivatives. Reactions with carbon dioxide give the N-nitroso-α-aminoacids which can be converted to α-acetoxynitrosamines by reaction with lead tetraacetate. This reaction sequence converted the non-carcinogen, dibenzylnitrosamine, to a highly mutagenic, derivative α-acetoxybenzyl-benzylnitrosamine. This reaction sequence has been used to prepare potential metabolic analogs of cyclic nitrosamines. Electrolytic oxidation of nitrosamines also appears to produce these α-oxygenated derivatives.

N-Nitrosocarbazole has been shown to nitrosate a variety of secondary amines by a photochemical mechanism. This reaction may provide a convenient, non-aqueous, synthetic route to N-nitrosamines.

Achiral N-nitrosamines have been shown to give induced circular dichroism on interaction with chiral alcohols. This may provide a method for studying complexes of N-nitrosamines with hydrogen bond donors.

Significance to Biomedical Research and the Program of the Institute: The investigations made under this contract contribute directly and indirectly to the addition of N-nitrosamines needed for biological research to the NCI Chemical Repository. These compounds are essential for an understanding of the metabolism and mode of carcinogenesis of nitrosamines. The synthetic studies may provide clues about environmental syntheses, and interconversion of nitrosamines as well as structural information may suggest the nature of interactions of nitrosamines with biological systems.

Proposed Course: The synthetic studies will be continued to prepare nitrosamines, labeled and unlabeled, as needed for research in carcinogenesis. The spectral studies will be continued to obtain information about the interaction of the nitrosamine function with other functions.

Date Contract Initiated: January 31, 1975

Current Annual Level: $2,544

SALK INSTITUTE FOR BIOLOGICAL STUDIES (N01-CP-75970)

Title of Project: Chemical Carcinogenesis and Control of the Cell Cycle

Contractor's Project Director: Dr. Robert W. Holley

Project Officer (NCI): Dr. Andrew C. Peacock

Objectives: The overall objective of this project is to understand the changes in growth control, particularly changes in control of the cell cycle, that take place during chemical carcinogenesis.

The project has been concerned particularly with the control of growth of benzo[a]pyrene-transformed 3T3 (BP3T3) cells. Among the factors that have been found to control the growth of these cells, is epidermal growth factor (EGF). In comparison with normal 3T3 cells, the chemically-transformed cells have been found to destroy EGF less rapidly, and as a result, the transformed cells proliferate more than the normal cells. Less EGF is bound to BP3T3 than to 3T3, and BP3T3 cells are more sensitive to the EGF that is bound.

The specific goal of the current contract is to measure the three components of total binding of EGF to the cells: (a) intact, surface-bound EGF, (b) intact, internalized EGF, and (c) degraded, internalized EGF. These data, in combination with earlier results, should permit a conclusion as to the nature of the changes that lead to relaxed growth control in this chemically-transformed cell.

Major Findings: Measurements of the three different components of the total binding of EGF have been carried out. After binding of ^{125}I-EGF to the cells, anti-serum to EGF has been used to remove and measure the intact surfacebound EGF. The internalized, degraded EGF was determined by measuring the acid-soluble ^{125}I present at the same time. The intact, internalized EGF was measured as the acid-insoluble ^{125}I present at the initial time that became acid-soluble during a subsequent incubation at 37°.

The measurements are largely complete but the data must be carefully analyzed. The preliminary conclusion is that the rates of internalization and degradation are approximately the same in the normal and transformed cells. Assuming this conclusion holds, it indicates that it is the lower affinity of BP3T3 cells for EGF plus the increase in sensitivity of BP3T3 cells to EGF that results in less destruction of EGF and greater proliferation of the chemically-transformed cells in a given amount of growth factor.

Significance to Biomedical Research and the Program of the Institute: This work provides the most detailed understanding obtained thus far of changes in growth control that result from transformation by a chemical carcinogen. Such knowledge will contribute eventually to rational approaches to treatment of malignancies.

Proposed Course: In the remaining months of the contract the data will be refined to the extent necessary and analyzed, and the results will be prepared for publication.

Date Contract Initiated: September 29, 1977

Current Annual Level: 0

SOUTHERN RESEARCH INSTITUTE (N01-CP-65740)

Title: Preparation of Carcinogens

Contractor's Project Director: Dr. Y. Fulmer Shealy

Project Officer (NCI): Dr. David Longfellow
Dr. A. R. Patel

Objectives: To synthesize, purify, and thoroughly characterize designated chemicals required for research on chemical carcinogenesis. The objectives are designed to serve a dual purpose: (1) to provide the carcinogenesis program with chemicals that are unavailable from other sources; (2) to provide well-characterized reference samples to investigators to eliminate variability in experimental results that might be caused by impurities in inadequately analyzed specimens from various sources.

Major Findings: During the past year, the following metabolites of the carcinogen 2-acetylaminofluorene (2-AAF) have been synthesized, purified, thoroughly characterized for identity and purity (m.p., UV, IR, MS, ^{1}H-NMR, ^{13}C-NMR, TLC, GC, HPLC), and sent to the Chemical Repository at IIT Research Institute: N-hydroxy-2-acetylaminofluorene, 1-hydroxy-2-acetylaminofluorene, 3-hydroxy-2-acetylaminofluorene, 5-hydroxy-2-acetylaminofluorene, 7-hydroxy-2-acetylaminofluorene, 9-hydroxy-2-acetylaminofluorene, and 3-methylthio-2-acetylaminofluorene.

The following N-nitrosamines have been prepared, thoroughly characterized for identity and purity by a variety of analytical and spectroscopic methods, and sent to the Chemical Repository at IIT Research Institute: N-nitrosodipropylamine, N-methyl-N-benzylnitrosamine, 2, 6-dimethylnitrosomorpholine, and α-ureidodimethylnitrosamine.

Significance to Biomedical Research and the Program of the Institute: The availability of well-characterized specimens of carcinogens, carcinogen metabolites, and potential carcinogens is essential to research on chemical carcinogenesis to assure that the results of biological and biochemical studies are not influenced by contaminants.

Proposed Course: The contract terminated on January 31, 1978. Through the auspices of a new Basic Ordering Agreement, the contractor will continue to synthesize and supply compounds as requested.

Date Contract Initiated: November 1, 1975

Current Annual Level: 0

STANFORD RESEARCH INSTITUTE (NO1-CP-65741)

Title: Preparation of Carcinogens

Contractor's Project Director: Dr. Elmer J. Reist

Project Officers (NCI): Dr. A. R. Patel
Dr. David Longfellow

Objectives: To synthesize sufficient quantities of carcinogens that will be characterized analytically for investigations of their biological action in causing cancer.

Major Findings: A number of carcinogens have been prepared and submitted to the Chemical Repository of the National Cancer Institute. They include 200 g of methyl acetoxymethyl nitrosamine, 1 kg of (4-hydroxybutyl)butyl nitrosamine, 2 g of 8-hydroxy-2-acetylaminofluorene, 10 g of N-hydroxy-4-acetylaminobiphenyl, 8.8 g of N-hydroxy-4-acetylaminostilbene, and 8.8 g of N-hydroxy-2-acetylaminophenanthrene.

Significance to Biomedical Research and the Program of the Institute: An estimated 80 to 90% of the cancers that occur in man are caused by carcinogenic substances in the environment. By studying these substances and their mechanisms of action, information may be derived that will lead to means of prevention of cancer in man.

Proposed Course: The contract concluded on February 28, 1978. Through the auspices of a new Basic Ordering Agreement, the contractor will continue to synthesize and supply compounds as requested.

Date Contract Initiated: November 26, 1975

Current Annual Level: $1,647

6. PUBLICATIONS OF THE CARCINOGENESIS RESEARCH PROGRAM

A. INTRAMURAL PROGRAM[1]

A1. Adams, R.A., DiPaolo, J.A., and Homburger, F.: The Syrian hamster in toxicology and carcinogenesis research. Cancer Res. (In Press).

A2. Angeles, R.M., Keefer, L.K., Roller, P.P., and Uhm, S.J.: Chemical models for possible artifactual nitrosamine formation in environmental analysis. IARC Scientific Publications. (In Press).

A3. Autrup, H., Harris, C.C., Fugaro, S., and Selkirk, J.K.: Effect of various chemicals on the metabolism of benzo(a)pyrene by cultured rat colon. Chem. Biol. Interact. 18: 337-347, 1977.

A4. Bergman, A., Mankowski, T., Chojnacki, T., De Luca, L.M., Peterson, E., and Dallner, G.: Glycosyl transfer from nucleotide sugars to C_{55}-,C_{85}-polyprenyl and retinylphosphates by microsomal subfractions and Golgi membranes of rat liver. Biochem. J. (In Press).

A5. Bowden, G.T., Gresselbach, B., and Fusenig, N.E.: Postreplication repair of DNA in ultraviolet light irradiated normal and malignantly transformed mouse epidermal cell cultures. Cancer Res. (In Press).

A6. Bowden, G.T., Hohneck, G., and Fusenig, N.E.: DNA excision repair in ultraviolet-irradiated normal and malignantly transformed mouse epidermal cell cultures. Cancer Res. 37: 1611-1617, 1977.

A7. Brown, C.A.: The cytochemical demonstration of beta-glucuronidase in colon neoplasms of rats exposed to azoxymethane. J. Histochem. Cytochem. 26: 22-27, 1978.

A8. Bustin, M.: Binding of E. coli RNA polymerase to chromatin subunits. Nucleic Acids Res. 5: 925-932, 1978.

A9. Bustin, M.: Histone antibodies: Structural probes for chromatin and chromosomes. In Busch, H. (Ed.): The Cell Nucleus. (In Press).

A10. Bustin, M.: Histone antibody and chromatin structure. In Sparkes, R.S., Comings, D.E., and Fox, C.F. (Eds.): Human Molecular Cytogenetics, Winter Symposia, New York, Academic Press, 1977, pp. 25-40.

A11. Bustin, M., Hopkins, R.B., and Isenberg, I.: Immunological relatedness of HMG chromosomal proteins from calf thymus. J. Biol. Chem. 253: 1694-1699, 1978.

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[1]Papers published exclusively from the Intramural Program's staff.

A12. Bustin, M., Kurth, P.D., Moudranakis, E.M., Goldblatt, D., Sperling, R., and Rizzo, W.: Immunological probes for chromatin structure. Cold Spring Harbor Symposia. (In Press).

A13. Bustin, M., Reeder, R.H., and McKnight, S.L.: Immunological cross reaction between calf and Drosophila histones. J. Biol. Chem. 252: 3099-3101, 1977.

A14. Bustin, M., Simpson, R.T., Sperling, R., and Goldblatt, D.: Molecular homogeneity of the histone content of HeLa chromatin subunits. Biochemistry 16: 5381-5385, 1977.

A15. Colburn, N.H.: Chemical transformation of epidermal cell cultures. In Saffiotti, U. and Autrup, H. (Eds.): In Vitro Carcinogenesis Guide to the Literature, Recent Advances and Laboratory Procedures. Carcinogenesis Program, DCCP, NCI: Technical Report Series No. 44. U.S. DHEW, PHS, NIH, NCI. DHEW Publ. No. (NIH) 78-844. Wash., D.C., U.S. Govt. Print. Off., 1978, pp. 57-64.

A16. Colburn, N.H., Vorder Bruegge, W.F., Bates, J.R., Gray, R.H., Rosseen, J.D., Kelsey, W.H., and Shimada, T.: In vitro transformation of mouse epidermal cells: Correlation of growth in semi-solid medium with tumorigenicity. Cancer Res. 38: 624-634, 1978.

A17. Colburn, N.H., Vorder Bruegge, W.F., Bates, J.R. and Yuspa, S.H.: Epidermal cell transformation in vitro. In Slaga, T.J., Sivak, A. and Boutwell, R.K. (Eds.): Mechanisms of Tumor Promotion and Cocarcinogenesis. (In Press).

A18. Day, R.S., III.: Human adenoviruses as DNA repair probes. In Nichols, W.W. and Murphy, D.G. (Eds.): DNA Repair Processes (Cellular Senescence and Somatic Cell Genetics Series). Miami, Symposia Specialists, 1977, pp. 119-145.

A19. Day, R.S., III.: Inducible error-prone repair and cellular senescence. In Nichols, W.W. and Murphy, D.G. (Eds.): DNA Repair Processes (Cellular Senscence and Somatic Cell Genetics Series). Miami, Symposia Specialists, 1977, pp. 217-223.

A20. Day, R.S., III.: UV-induced alleviation of K-specific restriction of phage lambda. J. Virol. 21: 1249-1251, 1977.

A21. Day, R.S., III and DiMattina, M.: Photodynamic action of chlorpromazine on adenovirus 5: Repairable damage and single-strand breaks. Chem. Biol. Interact. 17: 89-97, 1977.

A22. Day, R.S., III, Scudiero, D.A. and DiMattina, M.: Repair of DNA damaged by benzo(a)pyrene diol-epoxide I. In Ts'o, P.O.P. and Gelboin, H.V. (Eds.): Polycyclic Hydrocarbons and Cancer: Chemistry, Molecular Biology and Environment. (In Press).

A23. Day, R.S., III, Scudiero, D.A., and DiMattina, M.: Excision repair by human fibroblasts of DNA damaged by r-7, t-8-dihydroxy-t-9, 10-oxy-7,8,9,10 tetrahydrobenzo(a)pyrene. Mutat. Res. (In Press).

A24. Day, R.S., III and Ziolkowski, C.: Studies on UV-induced viral reversion, Cockayne's syndrome, and MNNG damage using adenovirus 5. In Hanawalt, P.C. and Friedberg, E.C. (Eds.): DNA Repair Processes. (In Press).

A25. De Luca, L.M.: The direct involvement of vitamin A in glycosyl transfer reactions of mammalian membranes. In Harris, R., Diczsalusy, E., Munson, P. and Glover, J. (Eds.): Vitamins and Hormones. New York, Academic Press, 1977, Vol. 35, pp. 1-57.

A26. De Luca, L.M.: Vitamin A. In De Luca, H.F. (Ed.): Fat-Soluble Vitamins. New York, Plenum Press, 1978, pp. 1-67.

A27. DiPaolo, J.A.: Chromosomal alterations in carcinogen transformed mammalian cells. In Ts'o, P.O.P. (Ed.): The Molecular Biology of the Mammalian Genetic Apparatus. Amsterdam, Associated Scientific Publishers, Vol. II, 1977, pp. 205-227.

A28. DiPaolo, J.A. and Donovan, P.J.: Transformation frequency of Syrian hamster cells and its modulation by UV-irradiation. In Hanna, M.G. (Ed.): International Conference on Ultraviolet Carcinogenesis. (In Press).

A29. DiPaolo, J.A. and Popescu, N.C.: Banding pattern analysis of initial structural chromosome alterations induced by N-methyl-N'-nitrosoguanidine in Syrian hamster cells. Mutat. Res. 44: 359-368, 1977.

A30. DiPaolo, J.A. and Popescu, N.C.: Cytogenetics of Syrian hamster and its relationships to in vitro neoplastic transformation. In Homburger, F. (Ed.): The Syrian Hamster in Toxicology and Carcinogenesis Research. (In Press).

A31. Doniger, J.: The mechanism of post-replication repair in mammalian cells. In Hanawalt, P.C., Friedberg, E.C. and Fox, C.F. (Eds.): DNA Repair Mechanisms. (In Press).

A32. Elgjo, K., Elgjo, R.F. and Hennings, H.: Morphology of mouse epidermal cells in vitro: A scanning electron microscopy study. Beitr. Pathol. 161: 122-130, 1977.

A33. Evans, C.H.: Neoplastic transformation by chemicals of guinea pig fetal cells in culture. In Saffiotti, U. and Autrup, H. (Eds.): In Vitro Carcinogenesis Guide to the Literature, Recent Advances and Laboratory Procedures. Carcinogenesis Program, DCCP, NCI: Technical Report Series No. 44. U.S. DHEW, PHS, NIH, NCI. DHEW Publ. No. (NIH) 78-844. Wash.,D.C., U.S. Govt. Print. Off., 1978, pp. 82-83.

A34. Evarts, R.P.: Effect of L-tryptophan on diethylnitrosamine and 3'-methyl-4-N-dimethylaminoazobenzene hepatocarcinogenesis. Food Cosmet. Toxicol. 15: 431-435, 1977.

A35. Evarts, R.P. and Brown, C.A.: Morphology of mammary gland, ovaries and pituitary gland of hydroxylamine fed C3H/HeN mice. Lab. Invest. 37: 53-63, 1977.

A36. Evarts, R.P., Brown, C.A. and Atta, G.J.: The effect of hydroxylamine on the morphology of the rat mammary gland and on the induction of mammary tumors by 7,12-dimethylbenz(a)anthracene. Lab. Invest. (In Press).

A37. Evarts, R.P. and Mostafa, M.H.: Dimethylnitrosamine demethylase activity of the rat liver microsomes after partial hepatectomy. Biochem. Pharmacol. (In Press).

A38. Frolik, C.A., Tavela, T.E., Peck, G.L., and Sporn, M.B.: High-pressure liquid chromatographic determination of 13-cis-retinoic acid and all-trans-retinoic acid in human plasma. J. Anal. Biochem. (In Press).

A39. Frolik, C.A., Tavela, T.E. and Sporn, M.B.: Separation of the natural retinoids by high pressure liquid chromatography. J. Lipid Res. 19: 32-37, 1978.

A40. Gaffield, W., Keefer, L.K. and Roller, P.P.: Synthesis of the selective bladder carcinogen, N-(n-butyl)-N-(3-carboxypropyl)nitrosamine (BCPN). Org. Prep. Pro. Int. 9: 49-52, 1977.

A41. Gelboin, H.V.: Cancer susceptibility and carcinogen metabolism. N. Engl. J. Med. 297: 384-386, 1977.

A42. Goldblatt, D., Sperling, R., and Bustin, M.: Heterogeneity in the interaction of chromatin subunits with anti-histone sera visualized by immunoelectron microscopy. Exp. Cell Res. 121: 1-14, 1978.

A43. Gothoskar, S.V., Benjamin, T., Roller, P.P. and Weisburger, E.K.: Metabolic fate of 2-aminoanthraquinone, a probable hepatocarcinogen and a nephrotoxic agent in the Fischer rat. In Proceedings of the Third International Symposium on the Detection and Prevention of Cancer. (In Press).

A44. Grantham, P.H., Mohan, L., Benjamin, T., Roller, P.P., Miller, J.R. and Weisburger, E.K.: Comparison of the metabolism of 2,4-toluenediamine in rats and mice. J. Toxicol. Environ. Health (In Press).

A45. Green, M.R.: Simultaneous differential staining of phosphoproteins, sialoglycoproteins, hyaluronic acid, sulfated glycosaminoglycans, proteins and nucleic acids in human breast tissue. J. Natl. Cancer Inst. (In Press).

A46. Green, M.R., Pastewka, J.V. and Peacock, A.C.: Bioassay for prolactin: Densitometric analysis on polyacrylamide gels of milk protein production by mammary explants in vitro. Endocrinology 101: 1784-1791, 1977.

A47. Harris, C.C. (Ed.): Pathogenesis and Therapy of Lung Cancer. (In Press).

A48. Harris, C.C.: Respiratory Carcinogenesis. In Straus, M. (Ed.): Lung Cancer: Clinical Diagnosis and Treatment. New York, Gruen and Stratton, Inc., 1977, pp. 1-17.

A49. Harris, C.C., Autrup, H. and Stoner, G.D.: Metabolism of benzo(a)pyrene by cultured human tissues and cells. In Ts'o, P.O.P. and Gelboin, H.V. (Eds.): Polycyclic Hydrocarbons and Cancer: Chemistry, Molecular Biology and Environment. (In Press).

A50. Hassell, J.R., Silverman-Jones, C.S. and De Luca, L.M.: The in vivo stimulation of mannose incorporation into mannosylretinylphosphate, dolichylmannosylphosphate, and specific glycopeptides of rat liver by high doses of retinyl palmitate. J. Biol. Chem. 253: 1627-1631, 1978.

A51. Hennings, H., Michael, D. and Patterson, E.: Croton oil enhancement of skin tumor initiation by N-methyl-N'-nitro-N-nitrosoguanidine: Possible role of DNA replication. Proc. Soc. Exp. Biol. Med. 158: 1-4, 1978.

A52. Hennings, H., Yuspa, S.H., Michael, D. and Lichti, U.: Modification of epidermal cell response to 12-O-tetradecanoyl-phorbol-13-acetate by serum level, cultured tempreature and pH. In Slage, T.J., Sivak, A. and Boutwell, R.K. (Eds.): Mechanisms of Tumor Promotion and Cocarcinogenesis. (In Press).

A53. Kakefuda, T. and Yamamoto, H.: Modification of DNA by benzo(a)pyrene metabolite, diol-epoxide 1. In Ts'o, P.O.P. and Gelboin, H.V. (Eds.): Polycyclic Hydrocarbons and Cancer: Chemistry, Molecular Biology and Environment. (In Press).

A54. Kakefuda, T. and Yamamoto, H.: Modification of DNA by the benzo(a)-pyrene metabolite diol-epoxide r-7, t-8-dihydroxy-t-9, 10-oxy-7,8,9,10-tetrahydrobenzo(a)pyrene. Proc. Nat. Acad. Sci. USA 75: 415-419, 1977.

A55. Kakefuda, T., Yamamoto, H. and Bak, T.: Enhancement of mutagenecity of benzo(a)pyrene metabolite by metal ions. Mutat. Res. (In Press).

A56. Kakunaga, T.: Chemical transformation of human and rodent cells. In Saffiotti, U. and Autrup, H. (Eds.): In Vitro Carcinogenesis Guide to the Literature, Recent Advances and Laboratory Procedures. Carcinogenesis Program, DCCP, NCI: Technical Report Series No. 44. U.S. DHEW, PHS, NIH, NCI. DHEW Publ. No. (NIH) 78-844. Wash., D.C., U.S. Govt. Print. Off., 1978, pp. 84-99.

A57. Kakunaga, T.: Factors affecting the polycyclic hydrocarbon-induced cell transformation. In Ts'o, P.O.P. and Gelboin, H.V. (Eds.): Polycyclic Hydrocarbons and Cancer: Chemistry, Molecular Biology and Environment. (In Press).

A58. Kakunaga, T.: Neoplastic transformation of human diploid cells by chemical carcinogens. Proc. Natl. Acad. Sci. USA (In Press).

A59. Kakunaga, T.: The transformation of human diploid cells by chemical carcinogens. In Hiatt, H.H., Watson, J.D. and Weinstein, J.A. (Eds.): Origins of Human Cancer. New York, Cold Spring Harbor Laboratory, 1977, Vol. 4, pp. 1537-1548.

A60. Kinshita, N. and Gelboin, H.V.: B-glucuronidase catalyzed hydrolysis of benzo(a)pyree-3-glucuronide and binding to DNA. Science 199: 307-309, 1978.

A61. Kraemer, K.H.: Progressive degenerative diseases associated with defective DNA repair xeroderma pigmentosum and ataxia telangiectasia. In Nichols, W.W. and Murphy, D.G. (Eds.): DNA Repair Processes. Miami, Symposia Specialists, 1977, pp. 37-71.

A62. Kraemer, K.H. and Weinstein, G.D.: Decreased thymidine incorporation in circulating leucocytes after treatment of psoriasis with psoraeen and long-wave ultraviolet light. J. Invest. Dermatol. 69: 211-214, 1977.

A63. Lichti, U., Yuspa, S.H. and Hennings, H.: Ornithine and S-adenosyl-methionine decarboxylases in mouse epidermal cell cultures treated with tumor promoters. In Slaga, T.J., Sivak, A. and Boutwell, R.K. (Eds.): Mechanisms of Tumor Promotion and Cocarcinogenesis. (In Press).

A64. Linnell, J.C., Quadros, E.V., Matthews, D.M., Morris, H.P. and Poirier, L.A.: Altered cobalamin distribution in rat hepatomas and in the livers of rats treated with diethylnitrosamine. Cancer Res. 37: 2975-2978, 1977.

A65. Martin, W.J., Gipson, T.G., Conliffe, M., Friedman, R.J., Dove, L. and Rice, J.M.: Common tumor-associated transplantation alloantigen detected on a proportion of lung tumors induced transplacentally in several strains of mice. Transplantation 24: 294-296, 1977.

A66. McKnight, S.L., Bustin, M. and Miller, D.L.: Ultrastructural and antigenic properties of metabolically active chromatin. Cold Spring Harbor Symposia. (In Press).

A67. Myhr, B.C. and DiPaolo, J.A.: Comparison of mutagenesis and malignant transformation in vitro with methyl methanesulfonate and N-acetoxy-2-acrtyl aminofluorene. Cancer Res. (In Press).

A68. Myhr, B.C. and DiPaolo, J.A.: Mutagenesis by N-acetoxy-2-acetyl aminofluorene of Chinese hamster V79 cells is unaffected by caffeine. Chem. Biol. Interact. (In Press).

A69. Nawata, H., Kato, K. and Ibayashi, H.: Age-dependent change of serum 5α-dihydrotestosterone and its relation to testosterone in man. Endocrinol. Jpn. 24: 41-45, 1977.

A70. Newton, D.L., Frolik, C.A., Roberts, A.B., Smith, J.M., Sporn, M.B., Nurrenbach, A. and Paust, J.: Biological activity and metabolism of the retinoid, axerophthene (vitamin A hydrocarbon). Cancer Res. (In Press).

A71. Petri, W.A. and Poirier, L.A.: A methionine-reversible folate deficiency in rats following the acute administration of diethylnitrosamine and α-naphthylisothiocyanate. Chem. Biol. Interact. 17: 1-7, 1977.

A72. Poirier, L.A. and Helmes, C.T.: Carcinogenesis by esters and benzylic alcohols. In Asher, I. and Zervos, C. (Eds.): Proceedings of the 2nd FDA Office of Science Summer Symposium. (In Press).

A73. Poirier, M.C. and DeCicco, B.T.: The kinetics of excision repair synthesis induced by N-acetoxy-2-acetylaminofluorene and ultraviolet irradiation in human diploid fibroblasts. J. Natl. Cancer Inst. 59: 339-343, 1977.

A74. Popescu, N.C., Turnbull, D. and DiPaolo, J.A.: Sister chromatid exchange and chromosome abberation using several carcinogens and non-carcinogens. J. Natl. Cancer Inst. 59: 289-293, 1977.

A75. Reese, D.H. and Friedman, R.D.: Suppression of dysplasia and hyperplasia by calcium in organ-cultured urinary bladder epithelim. Cancer Res. 38: 586-592, 1977

A76. Reese, D.H., Friedman, R.D., Gaffield, W. and Keefer, L.K.: Focal suppression and induction of hyperplasia by the bladder carcinogens butyl(4-hydroxybutyl)nitrosamine and butyl(3-carboxypropyl)-nitrosamine in organ-cultured rat bladder epithelim. J. Natl. Cancer Inst. 60: 219-223, 1978.

A77. Reese, D.H., Friedman, R.C. and Sporn, M.B.: Induction of hyperplasia and its suppression by hydrocortisone in organ-cultured rat urinary bladder. Cancer Res. 37: 1421-1427, 1977.

A78. Reitz, M.S., Saxinger, W.C., Ting, R.C., Gallo, R.C. and DiPaolo, J.A.: Lack of expression of type-C hamster virus after neoplastic transformation of hamster embryo fibroblasts by benzo(a)pyrene. Cancer Res. 37: 3583-3589, 1977.

A79. Rice, J.M.: Carcinogenesis: A late effect of irreversible toxic damage during development. Environ. Health Perspect. 18: 133-139, 1976.

A80. Rice, J.M.: Comparative aspects of transplacental carcinogenesis. Comp. Path Bull. 9 (4): 1 & 4, 1977.

A81. Rizzo, W.B. and Bustin, M.: Lectins as probes for chromatin structure. J. Biol. Chem. 252: 7062-7067, 1977.

A82. Saffiotti, U.: Carcinogenesis 1957-77: Notes for a historical review. J. Natl. Cancer Inst. 59: 617-622, 1977.

A83. Saffiotti, U.: Experimental approaches to the identification of environmental carcinogens. In Epstein, S.S. (Ed.): Environmental Determinants of Human Cancer. (In Press).

A84. Saffiotti, U.: Experimental identification of chemical carcinogens, risk evaluation and animal-to-human correlations. Environ. Health Perspect. (In Press).

A85. Saffiotti, U.: Eziologia ed etica: Problemi di una nuova tossicologia ambientale. Med. Lavoro. (In Press).

A86. Saffiotti, U.: Identifying and defining chemical carcinogens. In Hiatt, H.H., Watson, J.D. and Winsten, J.A.(Eds.): Origins of Human Cancer. New York, Cold Spring Harbor Laboratory, 1977, Vol. 4, pp. 1311-1326.

A87. Saffiotti, U.: In vitro carcinogenesis methods in relation to the development of carcinogenesis research. In Saffiotti, U. and Autrup, H. (Eds.): In Vitro Carcinogenesis Guide to the Literature, Recent Advances and Laboratory Procedures. Carcinogenesis Program, DCCP, NCI: Technical Report Series No. 44. U.S. DHEW, PHS, NIH, NCI. DHEW Publ. No. (NIH) 78-844. Wash.,D.C., U.S. Govt. Print. Off., 1978, pp. 1-20.

A88. Saffiotti, U.: Review of pesticides carcinogenesis data and regulatory approaches. IARC Scientific Publications. (In Press).

A89. Saffiotti, U.: Scientific bases of environmental carcinogenesis and cancer prevention: Developing an interdisciplinary science and facing its ethical implications. J. Toxicol. Environ. Health 2: 1435-1447, 1977.

A90. Saffiotti, U. and Autrup, H. (Eds.): In Vitro Carcinogenesis Guide to the Literature, Recent Advances and Laboratory Procedures. Carcinogenesis Program, DCCP, NCI: Technical Report Series No. 44. U.S. DHEW, PHS, NIH, NCI. DHEW Publ. No. (NIH) 78-844. Wash.,D.C., U.S. Govt. Print. Off., 1978, 316 pp.

A91. Saffiotti, U. and Harris, C.C.: Carcinogenesis studies on organ cultures of animal and human respiratory tissues. In Griffin, A.C. and Shaw, C.R. (Eds.): Carcinogens: Identification and Mechanisms of Action. (In Press).

A92. Saffiotti, U. and Page, N.P.: Releasing carcinogenesis test results: Timing and extent of report. J. Med. Pediatr. Oncol. 3: 159-167, 1977.

A93. Sato, M., De Luca, L.M. and Muto, Y.: Effects of exogenous retinol and retinoic acid on the biosynthesis of (^{14}C)mammose labelled glycolipids and glycoproteins in rat liver. J. Nutr. Sci. Vitaminol. (Toyko) 24: 9-23, 1978.

A94. Scudiero, D.A.: Repair deficiency in N-methyl-N'-nitro-N-nitrosoguanidine heated ataxia telangiectasia fibroblasts. In Hanawalt, P.C., Friedberg, E.C. and Fox, C.F. (Eds.): DNA Repair Mechanisms. (In Press).

A95. Selkirk, J.K.: Analysis of benzo(a)pyrene metabolism by high-pressure liquid chromatography. Adv. Chromatogr. (In Press).

A96. Selkirk, J.K.: Benzo(a)pyrene carcinogenesis: A biochemical selection mechanism. J. Toxicol. Environ. Health 2: 1245-1258, 1977.

A97. Sporn, M.B.: Prevention of epithelial cancer by vitamin A and its synthetic analogs (retinoids). In Hiatt, H.H., Watson, J.D. and Winsten, J.A. (Eds.): Origins of Human Cancer. New York, Cold Spring Harbor Laboratory, 1977, pp. 801-807.

A98. Sporn, M.B.: Vitamin A and its analogs (retinoids) in cancer prevention. In Winick, M. (Ed.): Nutrition and Cancer. New York, John Wiley & Sons, Inc., 1977, pp. 119-130.

A99. Sporn, M.B., Newton, D.L., Smith, J.M., Acton, N., Jacobson, A.R. and Brossi, A.: Retinoids and cancer prevention: The importance of the terminal group of the retinoid molecule in modifying activity and toxicity. In Griffin, A.C. and Shaw, C.R. (Eds.): Carcinogens: Identification and Mechanism of Action. (In Press).

A100. Stinson, S.F., Lilga, J.C., Reese, D.H., Friedman, R.D. and Sporn, M.B.: Quantitation with an automated image analyzer of nuclear-cytoplasmic changes induced by hydrocortisone in bladder epithelium. Cancer Res. 37: 1428-1431, 1977.

A101. Stinson, S.F. and Sporn, M.B.: Use of automated image analysis to detect precancerous changes in the bladder epithelium. J. Appl. Photogr. Eng. (In Press).

A102. Stinson, S.F. and Sporn, M.B.: Use of an automated image analyser to quantitate cellular hyperplasia in urinary bladder epithelium. J. Microsc. 109: 329-336, 1977.

A103. Stinson, S.F., Squire, R.A. and Sporn, M.B.: Pathology of esophageal neoplasms and associated proliferative lesions induced in rats by n-methyl-n-benzyl nitrosamine. J. Natl. Cancer Inst. (In Press).

A104. Sundaresan, P.R. and De Luca, L.M.: Vitamin A contents of rat intestinal epithelium and jejunal mucinous adenocarcinoma. J. Natl. Cancer Inst. 58: 1643-1645, 1977.

A105. Weisburger, E.K.: Carcinogenic natural products. Proceedings of the FDA Meeting on Structural Correlates of Carcinogenesis and Mutagenesis: A Guide to Testing Priorities? (In Press).

A106. Weisburger, E.K.: Carcinogenic natural products. In Carcinogenic Natural Products. (In Press).

A107. Weisburger, E.K.: Chemical carcinogenesis. Toxicology for Chemists. (In Press).

A108. Weisburger, E.K.: History of the Journal of the National Cancer Institute. J. Natl. Cancer Inst. 59: 601-604, 1977.

A109. Weisburger, E.K.: Industrial and environmental cancer risks. In Sax, N.I. (Ed.): Dangerous Properties of Industrial Materials. (In Press).

A110. Weisburger, E.K.: Mechanisms of chemical carcinogenesis. Ann. Rev. Pharmacol. Toxicol. (In Press).

A111. Weisburger, E.K.: Species specific biochemical pathways of malignant growth. In Kaiser, H.E. (Ed.): Patholigic Growth in Plants, Animals, and Man. (In Press).

A112. Weisburger, E.K.: Structure-activity relationships in carcinogenic drugs. In Bungaard, H., Jull, P. and Kofod, H.(Eds.): Alfred Benzon Symposium X - Drug Design and Adverse Reactions. Denmark, Munksgaard, 1977, pp. 246-260.

A113: Weisburger, E.K., Grantham, P.H. and Benjamin, T.: Separation and analysis of various carcinogenic aromatic amines and their metabolites. In Proceedings of the Third International Symposium on the Detection and Prevention of Cancer. (In Press).

A114. Wiebel, F.J., Brown, S., Minna, J.D. and Gelboin, H.V.: Assignment of a human gene for aryl hydrocarbon hydroxylase expression to chromosome 2. In Ullrich, V. (Ed.): Microsomes and Drug Oxidations. New York, Pergamon Press, 1977, pp. 426-434.

A115. Wiebel, F.J. and Gelboin, H.V.: Cutaneous carcinogenesis: Metabolic interaction of chemical carcinogens with skin. Handbook of Physiology. Baltimore, Williams & Wilkins, 1977, pp. 337-348.

A116. Wilson, M. and Poirier, L.A.: Increased methionine requirements of transformed liver cells in vitro. Exp. Cell Res. 111: 397-400, 1978.

A117. Yang, S.K., McCourt, D.W. and Gelboin, H.V.: The mechanism of hydrolysis of the non-K-region benzo(a)pyrene diol-epoxide r-7, t-8-dihydroxy-t-9, 10-oxy-7,8,9,10-tetrahydrobenzo(a)pyrene. J. Am. Chem. Soc. 99: 5131-5134, 1977.

A118. Yang, S.K., McCourt, D.W., Leutz, J.C. and Gelboin, H.V.: Benzo(a)pyrene diol-epoxides: Mechanism of enzymatic formation and optically active intermediates. Science 196: 1199-1201, 1977.

A119. Yang, S.K., McCourt, D.W., Gelboin, H.V., Miller, J.R. and Roller, P.P.: Stereochemistry of the hydrolysis products and their acetonides of two steroisometic benzo(a)pyrene-7,8-diol-9,10-epoxides. J. Am. Chem. Soc. 99: 5124-5130, 1977.

A120. Yang, S.K., Roller, P.P. and Gelboin, H.V.: Benzo(a)pyrene metabolism: Mechanism in the formation of epoxides, phenols, dihydrodiols and the 7,8-diol-9,10-epoxides. In Jones, P.W. and Freudenthal, R.I.: (Eds.): Carcinogenesis, Polycyclic Aromatic Hydrocarbons. New York, Raven Press, 1978, Vol. 3, pp. 285-301.

A121. Yang, S.K., Roller, P.P. and Gelboin, H.V.: Enzymatic mechanism of benzo(a)pyrene conversion to phenols and diols and an improved high pressure liquid chromatographic separation of benzo(a)pyrene derivatives. Biochemistry 16: 3680-3687, 1977.

A122. Yang, S.K., Roller, P.P. and Gelboin, H.V.: Mechanism of microsomal activation of benzo(a)pyrene to diol-epoxides: The separation and characterization of intermediates and products. In Ullrich, V., Roots, I., Hildebrandt, A., Estabrook, R.W. and Conney, A.H. (Eds.): *Third International Symposium on Microsomes and Drug Oxidations*. New York, Pergamon Press, 1977, pp. 403-410.

A122. Yuspa, S.H.: Mouse epidermal cell cultures as an in vitro model for the study of chemical carcinogenesis. In Saffiotti, U. and Autrup, H. (Eds.): In Vitro Carcinogenesis Guide to the Literature, Recent Advances and Laboratory Procedures. Carcinogenesis Program, DCCP, NCI: Technical Report Series No. 44. U.S. DHEW, PHS, NIH, NCI. DHEW Publ. No. (NIH) 78-844. Wash.,D.C., U.S. Govt. Print. Off., 1978, pp. 47-56.

B. COLLABORATIVE PROGRAM[1]

B1. Alexandrov, K. and Thompson, M.H.: Influence of inducers and inhibitors of mixed-function oxidases on benzo(a)pyrene binding to the DNA of rat liver nuclei. Cancer Res. 37: 1443-1449, 1977.

B2. Althoff, J. and Grandjean, C.: In vivo studies in hamsters--a transplacental bioassay of 10 nitrosamines. Natl. Cancer Inst. Monogr. (In Press).

B3. Althoff, J., Grandjean, C. and Gold, B.: Carcinogenic effect of subcutaneously administered N-nitroso-2,6-dimethylmorpholine in Syrian golden hamsters. J. Natl. Cancer Inst. 60: 197-199, 1978.

B4. Althoff, J., Grandjean, C. and Gold, B.: Diallylnitrosamine: A potent respiratory carcinogen in Syrian golden hamsters: Brief communication. J. Natl. Cancer Inst. 59: 1569-1571, 1977.

B5. Althoff, J., Grandjean, C. and Gold, B.: The effect of 1-methoxypropylpropylnitrosamine in Syrian golden hamsters. Z. Krebsforsch 90: 215-219, 1977.

B6. Althoff, J., Grandjean, C., Marsh, S., Pour, P. and Takahashi, M.: Transplacental effects of nitrosamines in Syrian hamsters. II. Nitrosopiperidine. Z. Krebsforsch 90: 71-77, 1977.

B7. Althoff, J., Grandjean, C. and Pour, P.: Transplacental effect of nitrosamines in Syrian hamsters. IV. Metabolites of dipropyl- and dibutylnitrosamine. Z. Krebsforsch 90: 119-126, 1977.

B8. Althoff, J., Grandjean, C., Pour, P. and Bertram, B.: Comparison of the effect of β-oxidized dipropylnitrosamine metabolites administered at equimolar doses to Syrian hamsters. Z. Krebsforsch 90: 141-148, 1977.

B9. Althoff, J., Grandjean, C., Pour, P. and Gold, B.: Local and systemic effects of 1-acetoxypropylpropylnitrosamine in Syrian golden hamsters. Z. Krebsforsch 90: 127-140, 1977.

B10. Althoff, J. and Lijinsky, W.: Urinary bladder neoplasms in Syrian hamsters after administration of N-nitroso-N-methyl-n-dodecylamine. Z. Krebsforsch 90: 227-231, 1977.

B11. Althoff, J., Mohr, U. and Chesterman, F.C.: Tumors of the urinary tract. IARC Scientific Publications (In Press).

B12. Althoff, J., Pour, P., Grandjean, C. and Marsh, S.: The transplacental effect of nitrosamines in Syrian hamsters. III. Dimethyl- and dipropylnitrosamine. Z. Krebsforsch 90: 79-86, 1977.

---

[1]Papers published exclusively from the Collaborative Program staff

B13. Andrews, A.W., Thibault, L.H. and Lijinsky, W.: The relationship between carcinogenicity and mutagenicity of some polynuclear hydrocarbons. Mutat. Res. (In Press).

B14. Andrews, A.W., Thibault, L.H. and Lijinsky, W.: The relationship between mutagenicity and carcinogenicity of some nitrosamines. Mutat. Res. (In Press).

B15. Arai, Y., Suzuki, Y. and Nishizuka, Y.: Hyperplastic and metaplastic lesions in the reproductive tract of male rats induced by neonatal treatment with diethylstilbestrol. Virchows Arch. Pathol. Anat. 376: 21-28, 1977.

B16. Archer, M.C. and Wishnok, J.S.: Quantitative aspects of human exposure to nitrosamines. Food Cosmet. Toxicol. 15: 233-235, 1977.

B17. Archer, M.C., Yang, H.S. and Okun, J.D.: Acceleration of nitrosamine formation at pH 3.5 by microorganisms. In Walker, E.A. and Griciute, L. (Eds.): Environmental N-Nitroso Compounds Analysis and Formation. IARC Scientific Publications. (In Press).

B18. Augenlicht, L.H.: Digestion of RNA in chromatin. In Stein, G., Stein, J. and Kleinsmith, L. (Eds.): Methods in Cell Biology, XIV Methods in Chromosomal Protein Research. New York, Academic Press, 1977, pp. 467-472.

B19. Augenlicht, L.H. and Lipkin, M.: Serum stimulation of human fibroblasts: Effect on nonhistones of nuclear ribonucleoprotein and chromatin. J. Cell Physiol. 92: 129-136, 1977.

B20. Aviv, H., Bastos, R.N. and Volloch, Z.: Globin mRNA biosynthesis in erythroid cells. Oji International Seminar on Genetic Aspects of Friend Virus and Friend Cells. (In Press).

B21. Aviv, H. and Kaminchik, J.: Biosynthesis of C-type viral RNA in Friend erthroleukemic cells. Oji International Seminar on Genetic Aspects of Friend Virus and Friend Cells. (In Press).

B22. Baird, W.M., Chern, C.J. and Diamond, L.: Formation of benzo(a)pyrene-glucuronic acid conjugates in hamster embryo cell cultures. Cancer Res. 37: 3190-3197, 1977.

B23. Baird, W.M. and Diamond, L.: The nature of benzo(a)pyrene-DNA adducts formed in hamster embryo cells depends on the length of time exposure to benzo(a)pyrene. Biochem. Biophys. Res. Commun. 77: 162-167, 1977.

B24. Baird, W.M. and Dipple, A.: Photosensitivity of DNA-bound 7,12-dimethylb-benz(a)anthracene. Int. J. Cancer 20: 427-431, 1977.

B25. Baker, J.J. and Cheng-Yu, M.: Character of the TEA detector in use with high pressure liquid chromatography. IARC Scientific Publications (In Press).

B26. Baker, R.M. and Ling, V.: Membrane mutants of mammalian cells in culture. In Korn, E.D. (Ed.): Methods in Membrane Biology. New York, Plenum Press, Vol. 9, 1977, pp. 337-384.

B27. Banerjee, M.R., Terry, P.M., Sakai, S. and Lin, F.K.: Regulation of messenger RNA and specific milk protein in mammary gland. J. Toxicol. Environ. Health 3: 281-308, 1977.

B28. Banerjee, M.R., Wood, B.G., Lin, F.K. and Crump, L.R.: Organ culture of whole mammary gland. Tissue Culture Assoc. Manual 2: 457-462, 1976.

B29. Barrett, J.C., Crawford, B.D., Grady, D.L., Hester, L.D., Jones, P.A., Benedict, W.F. and Ts'o, P.O.P.: Temporal acquisition of enhanced fibrinolytic activity by Syrian hamster embryo cells following treatment with benzo(a)pyrene. Cancer Res. 37: 3815-3823, 1977.

B30. Barrett, J.C., Crawford, B.D. and Ts'o, P.O.P.: Quantitation of fibrinolytic activity of Syrian hamster fibroblasts using ^{3}H-labeled fibrinogen prepared by reductive alkylation. Cancer Res. 37: 1182-1185, 1977.

B31. Basilico, C.: Selective production of cell cycle specific ts mutants. J. Cell Physiol. (In Press).

B32. Bastos, R.N. and Aviv, H.: Globin RNA precursor molecules: Biosynthesis and processing in erythroid cells. Cell 11: 641-650, 1977.

B33. Batzinger, R.P., Bueding, E., Reddy, B.S. and Weisburger, J.H.: Formation of mutagenic drug metabolite by intestinal microorganisms. Cancer Res. 38: 608-612, 1978.

B34. Baxter, G.C. and Stanners, C.P.: The effect of protein degradation on cellular growth characteristics. J. Cell Physiol. (In Press).

B35. Bech-Hansen, N.T., Sarangi, F., Sutherland, D.J.A. and Ling, V.: Rapid assays for evaluating the drug sensitivity of tumor cells. J. Natl. Cancer Inst. 59: 21-27, 1977.

B36. Becker, J.M., Ti, T.K., Moody, F.G., McGreevy, J.M. and Nelson, J.A.: A model of biliary pancreatic reflux. Surgery. (In Press).

B37. Berlinger, N.T., Lopez, C., Lipkin, M., Vogel, J.E. and Good, R.A.: Defective recognitive immunity in family aggregates of colon carcinoma. J. Clin. Invest. 59: 761-769, 1977.

B38. Berry, D.L., Slaga, T.J., Viaje, A., Wilson, N.M., DiGiovanni, J., Juchau, M.R. and Selkirk, J.K.: The effects of trichloropropene oxide on the ability of polyaromatic hydrocarbons and their "K-region" oxides to initiate skin tumors in mice and to bind to DNA in vitro. J. Natl. Cancer Inst. (In Press).

B39. Bibi, O., Schwartz, J., Eilam, Y., Shohami, E. and Cabantchik, Z.I.: Nucleoside transport in mammalian cell membranes. IV. Organomercurials and organomercurial-mercaptonucleoside complexes as probes for nucleoside transport systems in hamster cells. J. Membr. Biol. (In Press).

B40. Bigger, C.A.H., Tomaszewski, J.E. and Dipple, A.: Differences between products of binding of 7,12-dimethylbenz(a)anthracene to DNA in mouse skin and in a rat liver microsomal system. Biochem. Biophys. Res. Commun. 80: 229-235, 1978.

B41. Blankenstein, M.A., Broerse, J.J., Vries, J.B. de, Berg, K.J. van den, Knaan, S. and Molen, H.J. van der: The effect of subcutaneous administration of oestrogens on plasma oestrogen levels and tumour incidence in female rats. Eur. J. Cancer 13: 1437-1443, 1977.

B42. Blevins, R.D., Lee, M. and Regan, J.D.: Mutagenicity screening of five methyl carbamate insecticides and their nitroso derivatives using mutants of Salmonella Typhimurium LT2[1]. Mutat. Res. 56: 1-16, 1977.

B43. Blevins, R.D., Lijinsky, W. and Regan, J.D.: Nitrosated methylcarbamate insecticides: Effect on the DNA of human cells. Mutat Res. 44: 1-7, 1977.

B44. Bodell, W.J. and Banerjee, M.R.: DNA repair in normal and preneoplastic mammary tissues. Cancer Res. 38: 736-740, 1978.

B45. Boesel, R.W., Klipper, R.W. and Shain, S.A.: Induction of limited capacity androgen binding components in nuclear and cytoplasmic fractions of canine prostate. Endocr. Res. Commun. 4: 71-84, 1977.

B46. Boren, H.G. and Paradise, L.J.: Cytokinetics of lung. In Harris, C.C. (Ed.): Pathogenesis and Therapy of Lung Cancer. (In Press).

B47. Brake, E.T., Will, P.C. and Cook, J.S.: Characterization of HeLa 5'-nucleotidase: a stable plasma membrane marker. Membr. Biochem. (In Press).

B48. Brand, M.D. and Lehninger, A.L.: H+/ATP ratio during ATP hydrolysis by mitochondria: modification of the chemiosmotic theory. Proc. Nat. Acad. Sci. 74: 1955-1959, 1977.

B49. Brand, M.D., Lehninger, A.L. and Reynafarje, B.: The stoichiometric coupling of H+ efflux during electron transport to H+ influx during ATP synthesis by mitochondria. In Semenza, G. and Carafoli, E. (Eds.): Biochemistry of Membrane Transport. Berlin Heidelberg, Springer-Verlag, 1977, pp. 520-534.

B50. Broerse, J.J., Knaan, S., Bekkum, D.W. van, Hollander, C.F., Nooteboom, A.I. and Zweiten, M.J. van: Mammary carcinogenesis in rats after X- and neutron-irradiation and hormone administration. In Proceedings of the IAEA Symposium on the Late Biological Effects of Ionizing Radiation. (In Press).

B51. Brookes, P.: Mutagenicity of polycyclic aromatic hydrocarbons. Mutat. Res. 39: 257-284, 1977.

B52. Brookes, P.: Role of covalent binding in carcinogenicity. In Jallow, D.J., Kocsis, J.J., Snyder, R. and Vainio, H. (Eds.): Biological Reactive Intermediates. New York, Plenum Press, 1977, pp. 470-480.

B53. Buchwald, M.: Mutagenesis at the ouabain-resistance locus in human diploid fibroblasts. Mutat. Res. 44: 401-412, 1977.

B54. Bulay, O., Clayson, D.B. and Shubik, P.: Carcinogenic effects of niridazole in rats. Cancer Lett. (In Press).

B55. Bulay, O., Urman, H., Clayson, D.B. and Shubik, P.: Carcinogenic effects of niridazole on rodents infected with Schistosoma mansoni. J. Natl. Cancer Inst. 59: 1625-1630, 1977.

B56. Burke, M.D. and Prough, R.A.: Assay of underivatized biphenyl metabolites by high-pressure liquid chromatography. Anal. Biochem. 83: 466-473, 1977.

B57. Bynum, J., Regan, J.D. and Volkin, E.: RNA synthesis and processing as a measure of phenotypic variability in cytodifferentiation and neoplasia. J. Cell Physiol. 91: 1-14, 1977.

B58. Cabral, J.R.P., Shubik, P., Mollner, R. and Raitano, F.: Carcinogenic activity of hexachlorobenzene in hamsters. Nature 269: 510-511, 1977.

B59. Cardesa, A., Rustia, M. and Mohr, U.: Tumors of the nervous system in hamsters. In Manual of the Pathology of Tumors in Laboratory Animals: Tumors of Syrian Golden Hamsters. (In Press).

B60. Carlsen, S.A., Till, J.E. and Ling, V.: Modulation of membrane drug permeability in Chinese hamster ovary cells: Possible role for phosphorylation of surface flycoproteins. Biochim. Biophys. Acta 467: 238-250, 1977.

B61. Castagna, M. and Weisburger, J.H.: Nutrition et cancer: Ou est le danger? La Recherche 8: 286-288, 1977.

B62. Casto, B.C.: Enhancement of DNA viral transformation by chemical carcinogens and mutagens. In Saffiotti, U. and Autrup, H. (Eds.): In Vitro Carcinogenesis Guide to the Literature, Recent Advances and Laboratory Procedures. Carcinogenesis Program, DCCP, NCI: Technical Report Series No. 44. U.S. DHEW, PHS, NIH, NCI. DHEW Publ. No. (NIH) 78-844. Wash.,D.C., U.S. Govt. Print. Off., 1978, pp.226-230.

B63. Cavalieri, E., Mailander, P. and Pelfrene, A.: Carcinogenic activity of anthanthrene on mouse skin. Z. Krebsforsch 89: 113-118, 1977.

B64. Cavalieri, E., Roth, R., Althoff, J., Grandjean, C., Patil, K., Marsh, S. and McLaughlin, D.: Carcinogenicity and metabolic profiles of 3-methylcholanthrene oxygenated derivatives at the 1 and 2 positions. Chem. Biol. Interact. (In Press).

B65. Cavalieri, E., Roth, R., Liakus, S., Patil, K., Marsh, S., Grandjean, C., and Althoff, J.: Carcinogenicity and metabolic profiles of 6-substituted benzo(a)pyrene derivatives on mouse skin. Chem. Biol. Interact. (In Press).

B66. Cavalieri, E., Roth, R., Mailander, P. and Patil, K.: Isotope effect in the carcinogenicity of 3-methylcholanthrene after selective deuteration of the 1-methylene group. In Klein, E.R. and Klein, P.D. (Eds.): Proceedings of the Second International Conference on Stable Isotopes. ERDA Publ. No. 75-1027, Oak Brook, 1976, pp.19-31.

B67. Cavalieri, E., Roth, R., Rogan, E., Grandjean, C. and Althoff, J.: Mechanism of tumor initiation by polycyclic aromatic hydrocarbons. In Proceedings of the Second International Symposium on Polynuclear Aromatic Hydrocarbons. (In Press).

B68. Chevion, M., Blunberg, W.E. and Peisach, J.: Imidazole, the ligand trans to mercaptide in ferric cytochrome P-450; an EPR study of model compounds. J. Biol. Chem. 252: 3637-3645, 1977.

B69. Chevion, M., Stegeman, J.J., Peisach, J. and Blumberg, W.E.: EPR studies of hepatic microsomal cytochrome P-450 from a marine teleost fish. Life Sci. 20: 895-900, 1977.

B70. Chiba, Y., Chiba, K., Halberg, F. and Cutkomp, L.: Logitudinal evaluation of circadian rhythm characteristics and their circaseptan modulation in an apparently healthy couple. In McGovern, J., Reinberg, A. and Smolensky, M. (Eds.): Chronobiology in Allergy and Immunology. Springfield, Charles C. Thomas, 1977, pp.17-35.

B71. Chiu, J.F., Fujitani, H. and Hnilica, L.S.: Methods for selective extraction of chromosomal nonhistone proteins. Methods Cell Biol. 16: 283-296, 1977.

B72. Chiu, J.F., Hnilica, L.S., Belanger, L. and Morris, H.P.: Nuclear macromolecular changes in hepatomas. In Morris, H.P. and Criss, W.E. (Eds.): Morris Hepatomas: Mechanisms of Regulation. New York, Plenum Press, 1978, pp. 181-203.

B73. Chopra, D.P. and Wilkoff, L.J.: Induction of hyperplasia and anaplasia by carcinogens in organ cultures of mouse prostate. In Vitro 13: 260-267, 1977.

B74. Chopra, D.P. and Wilkoff, L.J.: Reversal by vitamin A analogues (retinoids) of hyperplasia induced by N-methyl-N'-nitro-N-nitrosoguanidine in mouse prostate organ cultures. J. Natl. Cancer Inst. 58: 923-930, 1977.

B75. Chu, C. and Murvish, S.S.: Effects of nine N-nitroso compounds on the specific radioactivity of liver proteins after injection of ^{14}C leucine into rats. Cancer Res. 37: 1565-1570, 1977.

B76. Chung, K.-T., Fulk, G.E. and Egan, M.: Reduction of azo dyes by intestinal anaerobes. Appl. Environ. Microbiol. (In Press).

B77. Chung, K.-T., Fulk, G.E. and Silverman, S.J.: Dietary effects on the composition of fecal flora of rats. Appl. Environ. Microbiol. 33: 654-659, 1977.

B78. Clayson, D.B.: Chairman's introduction: Perinatal carcinogenesis -- Biological curiosity or practical necessity. Natl. Cancer Inst. Monogr. (In Press).

B79. Clayson, D.B.: Environmental and industrial causes of bladder tumours. In Scientific Foundations of Urology, Vol. II. (In Press).

B80. Clayson, D.B.: Environmental carcinogens--chemical and physical factors. In Proceedings of the Eleventh Canadian Cancer Research Conference. Toronto, National Cancer Institute of Canada, 1976.

B81. Clayson, D.B.: Iatrogenic cancer. In Internal Medicine: 1976 Topics. Basel, Karger, 1977.

B82. Clayson, D.B.: Nutrition and experimental carcinogenesis. Columbia Series: New York Symposium on Nutrition and Cancer. (In Press).

B83. Clayson, D.B.: Principles underlying testing for carcinogenicity. Cancer Bull. 29: 161-166, 1977.

B84. Clayson, D.B.: Relationships between laboratory and human studies. J. Environ. Path. Toxicol. (In Press).

B85. Clayson, D.B.: The carcinogenic action of drugs in man and animals. Ecotoxicol. Env. Safety. (In Press).

B86. Conscience, J.-F., Miller, R.A., Henry, J. and Ruddle, F.H.: Acetylcholinesterase, carbonic anhydrase and catalase activity in Friend erythroleukemic cells, non-erythroid mouse cell lines and their somatic hybrids. Exp. Cell Res. 105: 401-412, 1977.

B87. Cortez, C. and Harvey, R.G.: Phenanthrene 9,10-oxide. Org. Syn. (In Press).

B88. Craighead, J.E. and Mossman, B.T.: Carcinoma induction by 3-methylcholanthrene in hamster tracheal tissue implanted in syngeneic animals. Prog. Exp. Tumor Res. (In Press).

B89. Creasia, D.A., Nettesheim, P. and Kim, J.C.S.: Stimulation of DNA synthesis in the lungs of hamsters exposed intermittently to nitrogen dioxide. J. Toxicol. Environ. Health 2: 1173-1181, 1977.

B90. Crerar, M.M., Andrews, S.J., David, E.S., Somers, D.G., Mandel, J.-L. and Pearson, M.L.: Amanitin binding to RNA polymerase II in α-amanitin-resistant rat myoblast mutants. J. Mol. Biol. 112: 317-329, 1977.

B91. Crerar, M.M. and Pearson, M.L.: RNA polymerase II regulation in α-amanitin-resistant rat myoblast mutants. Changes in wild-type and mutant enzyme levels during growth in α-amanitin. J. Mol. Biol. 112: 331-342, 1977.

B92. Cunha, G.R. and Lee, A.K.: The possible role of hemidesomosomes in neonatally estrogen-induced selection of permanently altered abnormal vaginal epithelium. J. Exp. Zool. 203: 361-370, 1978.

B93. Cunha, G.R., Lee, A.K. and Lung, B.: Electron microscopic observations of vaginal development in untreated and neonatally-estrogenized Balb/c Crgl mice. Am. J. Anat. (In Press).

B94. Cunha, G.R. and Lung, B.: The possible influences of temporal factors in androgenic responsiveness of urogenital tissue recombinants from wild-type and antrogen-insensitive (Tfm) mice. J. Exp. Zool. (In Press).

B95. Cunha, G.R., Lung, B. and Kato, K.: Role of epithelial-stromal interaction during the development and expression of ovary-independent vaginal hyperplasia. Dev. Biol. 56: 52-67, 1977.

B96. Darfler, F. and Tometski, A.M.: Application of light sensitive chemicals to probe biological processes. In Weinstein, B. (Ed.): Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins. (In Press).

B97. DeAngelo, A.B., Hudgins, W.R. and Kerby, S.B.: 7,12-Dimethylbenz(a)-anthracene-induced alteration of mammary epithelial cell RNA-synthetic patterns in rat strains of high and low tumor incidence. Cancer Res. 38: 384-389, 1978.

B98. DePierre, J.W. and Ernster, L.: The metabolism of polycyclic hydrocarbons and its relationship to cancer. Biochim. Biophys. Acta. (In Press).

B99. Deschner, E.E.: Colorectal biopsy and lavage for the evaluation of high-risk groups. Acta Endoscopica et Radiocinematographica. (In Press).

B100. Deschner, E.E.: Isotopically labeled colonic cytology in mice and men. Eur. J. Cancer. (In Press).

B101. Deschner, E.E. and Lipkin, M.: Alimentary cell kinetics: Generation and specialization. In Sircus, W. and Smith, A.N. (Eds.): Gastrointestinal Tract Cancer. (In Press).

B102. Deschner, E.E. and Lipkin, M.: Proliferation and differentiation of gastrointestinal cells in health and disease. In Lipkin, M. and Good, R.A. (Eds.): Gastrointestinal Tract Cancer. (In Press).

B103. Deschner, E.E. and Lipkin, M.: Proliferation of epithelial cells of the gastrointestinal tract in cancer and related diseases. In Glass, G.B.J. (Ed.): Progress in Gastroenterology. New York, Grune & Stratton, 1977, Vol. III, pp. 53-72.

B104. Deschner, E.E. and Long, F.C.: Acute and chronic effects of 1,2-dimethylhydrazine in mouse large bowel. In Nieburgs, H.E. (Ed.): Proceedings of the 3rd International Symposium on Detection and Prevention of Cancer. (In Press).

B105. Deschner, E.E. and Long, F.C.: Colonic neoplasms in mice produced with six injections of 1,2-dimethylhydrazine. Oncology 34: 255-257, 1977.

B106. Deschner, E.E., Winawer, S.J., Long, F.C. and Boyle, C.C.: ^{3}H-thymidine labeled colonic epithelial cells and mucosa in mice and men. Am. J. Dig. Dis. (In Press).

B107. Desrosiers, A.E., Kennedy, A.R. and Little, J.B.: Induction of lung cancer by ^{222}Rn decay products and benzo(a)pyrene. In Ryan, R.M., Mautuszek, J.M. and McLaughlin, J.E. (Eds.): Natural Radioactivity in Man's Environment. New York, Rensselaer Polytechnic Institute, 1976, pp. 182-191.

B108. Desrosiers, A.E., Kennedy, A.R. and Little, J.B.: ^{222}Radon daughter dosimetry in the Syrian golden hamster lung. Health Phys. (In Press).

B109. DeTraglia, M., Brand, J.S. and Tometski, A.M.: Characterization of azicobenzamidines as photoaffinity lables for trypsin. J. Biol. Chem. 253: 1846-1852, 1978.

B110. Diamond, L., O'Brien, T.G. and Rovera, G.: Inhibition of adipose conversion of 3T3 fibroblasts by tumour promoters. Nature 269: 247-249, 1977.

B111. Dipple, A., Baird, W.M., Bigger, C.A.H., Moschel, R.C. and Andrews, A.W.: DNA-carcinogen interactions. In Proceedings of 2nd Office of Sciences Summer Symposium of the U. S. Food and Drug Administration. (In Press).

B112. Dipple, A. and Roberts, J.J.: Excision of 7-bromomethylbenz(a)-anthracene-DNA adducts in replicating mammalian cells. Biochemistry 16: 1499-1503, 1977.

B113. Dipple, A. and Roberts, J.J.: Excision repair of DNA in 7-bromomethylbenz(a)anthracene-treated Chinese hamster and HeLa cells. Stud. Biophy. 61: 23-28, 1977.

B114. Dipple, A. and Nebzydoski, J.A.: Evidence for the involvement of a diol-epoxide in the binding of DMBA to DNA in cells in culture. Chem. Biol. Interact. 20: 63-75, 1978.

B115. Eilam, Y. and Bibi, O.: Distinct properties of uridine transport systems in growing, quiescent and serum stimulated hamster embryo cells. Biochim. Biophys. Acta 467: 51-64, 1977.

B116. Eilam, Y. and Cabantchik, Z.I.: Nucleoside transport in mammalian cell membranes. A specific inhibitory mechanism of high affinity probes. J. Cell Physiol. 92: 185-202, 1977.

B117. Engel, J.F., Sankaran, V., McCaustland, D.J., Kolwyck, K.C., Ebert, D.A. and Duncan, W.P.: Synthesis of ^{13}C-labeled benzo(a)pyrene derivatives. In Freudenthal, R.I. and Jones, P.W. (Eds.): Polycyclic Hydrocarbon Carcinogenesis: Chemistry, Biology and Environment. (In Press).

B118. Essigmann, J.M., Busby, W.F., Jr., and Wogan, G.N.: Quantitative determination of transfer RNA base composition using high-pressure liquid chromatography. Anal. Biochem. 81: 384-394, 1977.

B119. Essigmann, J.M., Croy, R.G., Nazdan, A.M., Busby, W.F., Jr., Reinhold, V.N., Buchi, G. and Wogan, G.N.: Structural identification of the major DNA adduct formed by aflatoxin B_1 in vitro. Proc. Natl. Acad. Sci. USA 74: 1870-1874, 1977.

B120. Estabrook, R.W., Werringloer, J., Capdevile, J. and Prough, R.A.: The oxidative metabolism of benzo(a)pyrene: The role of cytochrome P-450 and the microsomal electron transport system. In Ts'o, P.O.P. and Gelboin, H.V. (Eds.): Polycyclic Hydrocarbons and Cancer: Chemistry, Molecular Biology and Environment. (In Press).

B121. Farber, E.: Experimental liver carcinogenesis - A perspective. In Remmer, H. and Bolt, H.M. (Eds.): Primary Liver Tumours. (In Press).

B122. Farber, E.: Response of liver to carcinogens - A new analytical approach. In Farber, E. and Fisher, M.M. (Eds.): Toxic Injury of the Liver. (In Press).

B123. Farber, E., Cameron, R.G., Laishes, B., Lin, J-C., Medline, A., Ogawa, K. and Solt, D.B.: Physiological and molecular markers during carcinogenesis. In Proceedings of the 31st Annual M.D. Anderson Symposium on Fundamental Cancer Research. (In Press).

B124. Farber, E., Kawachi, T., Nagayo, T., Sugano, H., Sugimura, T. and Weisburger, J.H. (Eds.): Pathophysiology of Carcinogenesis in Digestive Organs. Baltimore, University Park Press, 1977, 411 pp.

B125. Farber, E. and Solt, D.B.: A new liver model for the study of promotion. In Proceedings of the Symposium on Mechanisms of Tumor Promotion and Cocarcinogenesis. (In Press).

B126. Farber, E., Solt, D.B., Cameron, R.G., Laishes, B., Ogawa, K. and Medline, A.: Newer insights into the pathogenesis of liver cancer. Am. J. Path. 89: 477-482, 1977.

B127. Fisher, P.B., Weinstein, I.B., Eisenberg, D. and Ginsberg, H.S.: Interactions between adenovirus, a tumor promoter and dimethyl ben(a)-anthracene in the transformation of rat embryo cell cultures. Proc. Natl. Acad. Sci. (In Press).

B128. Flintoff, W.F., Spindler, S.M. and Siminovitch. L.: Genetic characterization of methotrexate-resistant Chinese hamster ovary cells. In Vitro 12: 749-757, 1976.

B129. Fournier, R.E.K. and Ruddle, F.H.: Stable association of the human transgenome and host murine chromosomes demonstrated using trispecific microcell hybrids. Proc. Natl. Acad. Sci. USA 74: 3937-3941, 1977.

B130. Freeman, A.E., Lake, R.S., Igel, H.J., Gernand, L., Pezzutti, M.R., Malone, J.M., Mark, C. and Benedict, W.F.: Heteroploid conversion of human skin cells by methylcholanthrene. Proc. Natl. Acad. Sci. USA 74: 2451-2455, 1977.

B131. Freeman, A.E., Lake, R.S., Igel, H.J., Gernand, L., Pezzutti, M.R., Malone, J.M., Mark, C. and Benedict, W.F.: Heteroploid conversion of human skin cells by methylcholanthrene: Possible role of hydrocarbon metabolizing epithelial cells. In Saffiotti, U. and Autrup, H. (Eds.): In Vitro Carcinogenesis Guide to the Literature, Recent Advances and Laboratory Procedures. Carcinogenesis Program, DCCP, NCI: Technical Report Series No. 44. U.S. DHEW, PHS, NIH, NCI. DHEW Publ. No. (NIH) 78-844. Wash.,D.C., U.S. Govt. Print. Off., 1978, pp. 100-122.

B132. Fu, P.P. and Harvey, R.G.: (^{3}H)-7,12-Dimethylbenz(a)anthracene-5, 6-oxide. J. Labelled Compds. Radiopharm. 13: 619-621, 1977.

B133. Fu, P.P. and Harvey, R.G.: Dehydrogenation of polycyclic hydroaromatic compounds. Chem. Rev. (In Press).

B134. Fu, P.P. and Harvey, R.G.: Regiospecific catalytic hydrogenation of polynuclear hydrocarbons. Tetrahedron Lett. 6: 415-418, 1977.

B135. Fu, P.P. and Harvey, R.G.: Synthesis of the diols and diolepoxides of carcinogenic hydrocarbons. Tetrahedron Lett. 2059-2062, 1977.

B136. Fu, P.P., Harvey, R.G. and Beland, F.A.: Molecular orbital theoretical prediction of the isomeric products formed from reactions of arene oxides and related metabolites of polycyclic aromatic hydrocarbons. Tetrahedron. (In Press).

B137. Fu, P.P., Lee, H.M. and Harvey, R.G.: Novel synthesis of the dihydroarene precursors of carcinogenic arene dihydrodiols and diolepoxides. Tetrahedron Lett. 551-554, 1978.

B138. Fujita, S. and Peisach, J.: Cytochrome P-450 as hepatic microsomal azo reductase. Mol. Pharmacol. (In Press).

B139. Fujita, S. and Peisach, J.: Electron transfer between liver microsomal cytochrome b_5 and cytochrome P-450 in the azo reductase reaction. Biochem. Biophys. Res. Commun. 78: 328-335, 1977.

B140. Fujitani, H., Chiu, J.F. and Hnilica, L.S.: Specific nuclear antigens in Novikoff hepatoma. Proc. Natl. Acad. Sci. USA. (In Press).

B141. Gilboa, E., Soreq, H. and Aviv, H.: Initiation of RNA synthesis in isolated nuclei. Eur. J. Biochem. 77: 393-400, 1977.

B142. Gingell, R. and Pour, P.: Metabolism of the pancreatic carcinogen N-nitrosobis(2-oxopropyl)amine after oral and introperitoneal administration to hamsters. J. Natl. Cancer Inst. (In Press).

B143. Gluzman, Y., Davison, J., Oren, M. and Winocour, E.: Properties of permissive monkey cells transformed by UV-irradiated simian virus 40. J. Virol. 22: 256-266, 1977.

B144. Gluzman, Y., Kuff, E.L. and Winocour, E.: Recombination between endogenous and exogenous SV40 genes: I. Rescue of a SV40 temperature-sensitive mutant by passage in permissive transformed monkey lines. J. Virol. 24: 534-540, 1977.

B145. Bold, B. and Mirvish, S.S.: N-Nitroso derivatives of hydrochlorothiazide, niridazole and tolbutamide. Toxicol. Appl. Pharmacol. 40: 131-136, 1977.

B146. Gordon, R.E. and Lane, B.P.: Cytokinetics of rat tracheal epithelium stimulated by mechanical trauma. Cell Tissue Kinet. 10: 171-181, 1977.

B147. Gordon, R.E. and Lane, B.P.: Evidence for G_1 and G_2 arrested cells in normal rat tracheal epithelium. Cell Tissue Kinet. (In Press).

B148. Gordon, R.E., Lane, B.P. and Miller, F.: Electron microscope demonstration of tubulin in cilia and basal bodies of rat tracheal epithelium by the use of an antitubulin antibody. J. Cell Biol. 75: 586-592, 1977.

B149. Grandjean, C. and Althoff, J.: The significance of the hamster model in carcinogenesis testing. Prog. Exp. Tumor Res. (In Press).

B150. Green, J.D., Leong, B.K.J. and Laskin, S.: Inhaled benzene fetotoxicity in rats. Toxicol. Appl. Pharmacol. (In Press).

B151. Green, L.C., Hansen, T.J., Iwaoka, W. and Tannenbaum, S.R.: Specific detection systems for the chromatographic analysis of nitrosamines. In Krol, B. and Tinbergen, B.J. (Eds.): Proceedings of the Second International Symposium on Nitrite in Meat Products. Wageningen, Centre for Agricultural Publsihing and Documentation, 1976, pp. 145-153.

B152. Greenhouse, W.V.V. and Lehninger, A.L.: Magnitude of malate-asparate reduced nicotinamide adenine dinucleotide shuttle activity in intact respiring tumor cells. Cancer Res. 37: 4173-4181, 1977.

B153. Griesemer, R.A., Nettesheim, P., Martin, D.H., Jr. and Caton, J.R., Jr.: Quantitative exposure of grafted rat tracheas to 7,12-dimethylbenz(a) anthracene. Cancer Res. 37: 1266-1271, 1977.

B154. Groner, Y., Gilboa, E. and Aviv, H.: Methylation and capping of RNA polymerase II primary transcripts by HeLa nuclear homogenates. Isr. J. Med. Sci. 13: 933-938, 1977.

B155. Gudas, L.J., Cohen, A., Ullman, B. and Martin, D.W., Jr.: Analysis of adenosine-mediated pyrimidine starvation using cultured wild-type and mutant mouse T-lymphoma cells. Som. Cell Genet. (In Press).

B156. Gudas, L.J., Ullman, B., Cohen, A. and Martin, D.W., Jr.: Deoxyguanosine toxicity in mouse T-lymphosarcoma cells and its relationship to purine nucleoside phosphorylase associated immunodeficiency. Cell. (In Press).

B157. Guialis, A., Beatty, B.G., Ingles, C.J. and Crerar, M.M.: Regulation of RNA polymerase II activity in α-amanitin-resistant CHO hybrid cells. Cell 10: 53-60, 1977.

B158. Gupta, R.S., Chan, D.Y.H. and Siminovitch, L.: Evidence obtained by segregation analysis for functional hemizygosity at the Emt^r locus in CHO cells. Cell (In Press).

B159. Gupta, R.S., Flintoff, W.F. and Siminovitch, L.: Purification and properties of dihydrofolate reductase from methotrexate-sensitive and -resistant Chinese hamster ovary cells. Can. J. Biochem. 55: 445-452, 1977.

B160. Gupta, R.S. and Siminovitch, L.: An in vitro analysis of the dominance of emetine sensitivity in Chinese hamster ovary cell hybrids. J. Biol. Chem. (In Press).

B161. Gupta, R.S. and Siminovitch, L.: Diptheria toxin resistant mutants of CHO cells affected in protein synthesis. A novel phenotype. Som. Cell Genet. (In Press).

B162. Gupta, R.S. and Siminovitch, L.: Genetic and biochemical characterization of mutants of CHO cells resistant to the protein synthesis inhibitor trichodermin. Som. Cell Genet. 4: 355-374, 1978.

B163. Gupta, R.S. and Siminovitch, L.: The isolation and characterization of mutants of human diploid fibroblasts resistant to diphtheria toxin. Proc. Natl. Acad. Sci. USA (In Press).

B164. Gurtoo, H., Minowada, J., Paigen, B., Parker, N.B. and Hayner, N.T.: Factors influencing the measurement of aryl hydrocarbon hydroxylase in cultured human lymphocytes. J. Natl. Cancer Inst. 59: 787-789, 1977.

B165. Halberg, E., Halberg, F., Haus, E., Cornelissen, G., Wallach, L.A., Garcia Sainz, M., Simpson, H.W. and Shons, A.R.: Toward a chronobiopsy: Part I. A chronobiologic case report and a thermopsy complementing the biopsy. Chronobiologia (In Press).

B166. Halberg, E., Halberg, J., Halberg, F. and Halberg, F.: Infradian rhythms in oral temperature before human menarche. In Proceedings of the Symposium on the Menstrual Cycle. (In Press).

B167. Halberg, F., Carandente, E., Cornelissen, G. and Katinas, G.S.: Glossary of chronobiology. Chronobiologia (Supplement 1) 4: 1-189, 1977.

B168. Halberg, F., Gupta, B.D., Haus, E., Halberg, E., Deka, A.C., Nelson, W., Sothern, R.B., Cornelissen, G., Lee, J.K., Lakatua, D.J., Scheving, L.E. and Bunrs, E.R.: Steps toward a cancer chronopolytherapy. In Proceedings of the XIVth International Congress of Therapeutics. Paris, L'Expansion Scientifique Francaise, 1977, pp. 151-196.

B169. Halberg, F., Lee, J.K. and Nelson, W.: Time-qualified reference intervals -- chronodesms. Experientia. (In Press).

B170. Halberg, F. and Nelson, W.: Biorhythms and aging. In Proceedings of the Symposium on Aging. Advances in Experimental Medicine and Biology. (In Press).

B171. Hansen, T., Iwaoka, W., Green, L. and Tannenbaum, S.R.: Analysis of N-nitrosoproline in raw bacon. Further evidence that nitrosoproline is not a major precursor of nitrosopyrrolidine. J. Agri. Food Chem. 25: 1423-1426, 1977.

B172. Hardy, K., Fujitani, H., Chiu, J.F. and Hnilica, L.S.: Functional organization of chromatin. In O'Malley, B.W. and Birnbaumer, L. (Eds.): Hormone Receptors, Steriod Hormones. New York, Academic Press, Vol. I, 1977, pp. 151-194.

B173. Harris, J.F. and Whitmore, G.F.: Segregation studies in CHO hybrid cells: 1. Spontaneous and mutagen-induced segregation events of two recessive drug resistant loci. Som. Cell Genet. 3: 173-193, 1977.

B174. Harrison, S.D., Jr., Hixson, E.J., Burdeshaw, J.A. and Denine, E.P.: Effect of aspirin administration on retinoic acid toxicity in mice. Nature 269: 511-512, 1977.

B175. Hart, R.W., Setlow, R.B. and Woodhead, A.D.: Evidence that pyrimidine dimers in DNA can give rise to tumors. Proc. Natl. Acad. Sci. 74: 5574-5578, 1977.

B176. Harvey, R.G. and Cho, H.: Efficient resolution of the dihydrodiol derivatives of benzo(a)pyrene by high pressure liquid chromatography of the related (-)-dimenthoxyacetates. Anal. Biochem. 80: 540-546, 1977.

B177. Harvey, R.G. and Fu, P.P.: Synthesis and reactions of diolepoxides and related metabolites of carcinogenic hydrocarbons. In Ts'o, P.O.P. and Gelboin, H.V. (Eds.): Polycyclic Hydrocarbons and Cancer: Chemistry, Molecular Biology and Environment. (In Press).

B178. Harvey, R.G. and Sukumaran, K.: Synthesis of the a-ring dihydrodiols and anti-diolepoxides of benz(a)anthracene. Tetrahedron Lett. 2387-2390, 1977.

B179. Heckman, C.A., Marchok, A.C. and Metteshein, P.: Respiratory tract epithelium in primary culture: Concurrent growth and differentiation during establishment. J. Cell Sci. (In Press).

B180. Heichal, I., Bibi, O., Katz, J. and Cabantchik, Z.I.: Nucleoside transport in mammalian cell membrances. III. Kinetic and chemical modification studies of cytosine-arabinoside and uridine transport in hamster cells in culture. J. Membr. Biol. (In Press).

B181. Hixson, E.J. and Denine, E.P.: Comparative subacute toxicity of all-trans- and 13-cis-retinoic acid in Swiss mice. Toxicol. Appl. Pharmacol. (In Press).

B182. Hnilica, L.S.: The structure and biological function of histones. 2nd Edition. (In Press).

B183. Hnilica, L.S., Chiu, J.F., Hardy, K. and Fujitani, H.: Chromosomal proteins in differentiation. In Saunders, G. (Ed.): Cell Differentiation and Neoplasia. (In Press).

B184. Hnilica. L.S., Chiu, J.F., Fujitani, H., Hardy, K. and Briggs, R.C.: Antigenic properties of non-histone proteins in chromatin. In Whelan, W.J. and Schultz, J. (Eds.): Differentiation and Development. (In Press).

B185. Hnilica. L.S., Chiu, J.F., Hardy, K., Fujitani, H. and Briggs, R.C.: Antibodies of nuclear chromatin fractions. In Busch, H. (Ed.): The Cell Nucleus. (In Press).

B186. Hnilica, L.S., Grimes, S.R. and Chiu, J.F.: Electrophoretic fractionation of histones utilizing starch gels and sodium dodecyl sulfate urea gels. Methods Cell Biol. (In Press).

B187. Hsu, W.T., Harvey, R.G., Lin, E.J. and Weiss, S.B.: A bacteriophage system for the screening and study of biologically active polycyclic aromatic hydrocarbons and related compounds. Proc. Natl. Acad. Sci. USA 74: 1378-1382, 1977.

B188. Hsu, W.T., Lin, E.J., Harvey, R.G. and Weiss, S.B.: On the mechanism of ϕX 174 DNA inactivation by benzo(a)pyrene-7,8-dihydrodiol-9,10-oxide. Proc. Natl. Acad. Sci. USA 74: 3335-3339, 1977.

B189. Huberman, E. and Sachs, L.: DNA binding and its relationship to carcinogenesis by different polycyclic hydrocarbons. Int. J. Cancer 19: 122-127, 1977.

B190. Indo, K. and Wilson, R.B.: Fetal rat keratinizing epidermal cells in culture: Effects of long-term treatment by benzo(a)pyrene on their growth characteristics. J. Natl. Cancer Inst. 59: 867-880, 1977.

B191. Iqbal, Z.M. and Epstein, S.S.: Evidence of DNA repair in the guinea pig pancreas, in vivo and in vitro, following exposure to N-methyl-N-nitrosourethane. Chem. Biol. Interact. 20: 77-87, 1978.

B192. Issaq, H.J.: The determination of lead in aqueous solutions by the delves cup technique and flameless atomic absorption spectrometry. In Kirchhoff, W.H. (Ed.): Methods and Standards for Environmental Measurement. U.S. Dept. of Commerce, NBS Spec. Publ. No. 464, Wash.,D.C., U.S. Govt. Print. Off., 1977, pp. 497-500.

B193. Issaw, H.J., Chan, J.A. and Barr, E.W.: Thin-layer chromatographic determination of the antitumor agent 3,6-bis(5-chloro-2-piperidinyl)-2,5-piperazinedione in fermentation broth. J. Chromatogr. (In Press).

B194. Issaw, H.J., Schroer, J.A. and Barr, E.W.: A direct on-line thin layer chromatography/mass spectrometry coupling system. Chem. Instrumen. 8(1): 51-53, 1977.

B195. Issaq, H.J., Shaikh, B., Pontzer, N.J. and Barr, E.W.: Correlation of TLC, HPTLC and PMD results and their transfer to PHLC systems. J. Liq. Chromatogr. (In Press).

B196. Issaq, H.J. and Young, R.M.: Peak area vs. peak height in flameless atomic absorption measurements. Appl. Spectros. 31: 171-173, 1977.

B197. Ivanovic, V., Geacintov, N., Yamasaki, H. and Weinstein, I.B.: DNA and RNA adducts formed in hamster embryo cell cultures exposed to benzo(a)-pyrene. Biochemistry (In Press).

B198. Jakobsson, S.W., Okita, R.T., Prough, R.A., Mock, N.I., Buja, L.M., Graham, J.W., Petty, C.A. and Masters, B.S.S.: Studies on the cytochrome P-450 dependent monooxygenase systems of human liver, lung and kidney microsomes. In Gut, I. (Ed.): In Vivo Aspects of Biotransformation and Toxicity of Industrial and Environmental Xenobiotics. (In Press).

B199. Jamasbi, R.J. and Nettesheim, P.: Demonstration of cellular and humoral immunity to transplantable carcinomas derived from the respiratory tract of rats. Cancer Res. 38: 261-267, 1977.

B200. Jamasbi, R.J. and Nettesheim, P.: Demonstration of cross-reacting tumor rejection antigens in chemically induced respiratory tract carcinomas. Cancer Res. 37: 4059-4063, 1977.

B201. Jamasbi, R.J. and Nettesheim, P.: Increase in immunogenicity of a pulmonary squamous cell carcinoma propagated in vitro. Int. J. Cancer 20: 817-823, 1977.

B202. Jamasbi, R.J. and Nettesheim, P.: Nonimmunological enhancement of tumor transplantability in X-irradiated host animals. Br. J. Cancer (In Press).

B203. Jamasbi, R.J., Nettesheim, P. and Kennel, S.J.: Detection of circulating tumor antigens in mice carrying a highly metastatic pulmonary squamous cell carcinoma. Int. J. Cancer (In Press).

B204. Janss, D.H. and Ben, T.L.: Age related modification of 7,12-dimethylbenz(a)anthracene binding to rat mammary gland DNA. J. Natl. Cancer Inst. 60: 173-177, 1978.

B205. Jennette, K.W., Jeffrey, A.M., Blobstein, S.H., Beland, F.A., Harvey, R.G. and Weinstein, I.B.: Characterization of nucleoside adducts from the in vitro reaction of benzo(a)pyrene-7,8-dihydrodiol-9,10-oxide or benzo(a)-pyrene-4,5-oxide with nucleic acids. Biochemistry 16: 932-938, 1977.

B206. Jernstrom, B., Vadi, H. and Orrenius, S.: Formation of DNA-binding products from isolated benzpyrene metabolites in rat liver nuclei. Chem. Biol. Interact. (In Press).

B207. Johannesen, K.A.M. and DePierre, J.W.: Measurement of cytochrome P-450 in the presence of large amounts of contaminating hemoglobin and methemoglobin. Anal. Biochem. (In Press).

B208. Juliano, R.L. and Ling, V.: A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. Biochim. Biophys. Acta 455: 152-162, 1976.

B209. Katz, G.V. and Laskin, S.: Effect of irritant atmospheres on macrophage behavior. In Proceedings of the 16th Annual Hanford Biology Symposium on Pulmonary Macrophage and Epithelial Cells. (In Press).

B210. Kawasaki, T., Matsuoka, M., Halberg, E. and Halberg, F.: Circadian and circatrigintan rhythms in pulse, oral temperature and blood pressure of a clinically healthy Japanese woman. Chronobiologia (In Press).

B211. Kellermann, G.: Hereditary factors in human cancer. In Hiatt, H.H., Watson, J.D. and Winsten, J.A. (Eds.): Origins of Human Cancer. New York, Cold Spring Harbor Laboratory, Vol. 4, 1977, pp. 837-845.

B212. Kellermann, G.: Metabolic and enzymatic factors in tumor susceptibility. In Proceedings of the Third International Symposium on Detection and Prevention of Cancer. (In Press).

B213. Kellermann, G. and Brandenburg, J.: Aryl hydrocarbon hydroxylase induction in laryngeal carcinomas. Arch. Otolaryngol. (In Press).

B214. Kellermann, G. and Luyten-Kellermann, M.: Relationship of Benzo(a)-pyrene metabolism with plasma elimination rates of phenocetin, acetanilide and throphylline in man. Pharmacology (In Press).

B215. Kellermann, G., Luyten-Kellermann, M., Jett, J.R., Moses, H.L. and Fontana, R.S.: Aryl hydrocarbon hydroxylase in man and lung cancer. Hum. Genet. (In Press).

B216. Kelsey, M.I., Molina, J.E. and Hwang, K.-K.: A comparison of lithocholic acid metabolism by intestinal microflora in subjects of high and low-risk colon cancer populations. Front. Gastrointest. Res. (In Press).

B217. Kelsey, M.I., Muschik, G.M. and Sexton, S.A.: The metabolism of lithocholic acid-3α-sulfate by human intestinal microflora. Lipids 13: 152-157, 1978.

B218. Kelsey, M.I., Sexton, S., Fulk, G.E. and Chung, K.-T.: Unaltered metabolism of taurolithocholic acid with changes in composition of rat intestinal microflora. Appl. Environ. Microbiol. 33: 1016-1018, 1977.

B219. Kennedy, A.R., Desrosiers, A.E., Terzaghi, M. and Little, J.B.: Morphometric and histological analysis of the lungs and Syrian golden hamsters. J. Anat. (In Press).

B220. Kennedy, A.R. and Little, J.B.: Histochemistry of normal lungs and ^{210}Po-induced pulmonary tumors in hamsters. Acta Histochem. 58: 353-359, 1977.

B221. Kennedy, A.R. and Little, J.B.: Radiation carcinogenesis in the respiratory tract. In Harris, C.C. (Ed.): Pathogenesis and Therapy of Lung Cancer. (In Press).

B222. Kennedy, A.R. and Little, J.B.: Respiratory system differences between hamsters and other species which have relevance to lung carcinogenesis. Prog. Exp. Tumor Res. (In Press).

B223. Kennedy, A.R., McGandy, R.B. and Little, J.B.: Histochemical, light and electron microscopic study of polonium-210 induced peripheral tumors in hamster lungs: Evidence implicating the Clara cell as the cell of origin. Eur. J. Cancer 13: 1325-1340, 1977.

B224. Kennedy, A.R., McGandy, R.B. and Little, J.B.: Morphologic and histochemical characteristics of cell lines derived from hamster lung combined epidermoid and adenocarcinomas. Eur. J. Cancer 13: 1341-1350, 1977.

B225. Kennedy, A.R., McGandy, R.B. and Little, J.B.: Pathogenesis of ^{210}Po-induced lung tumors in Syrian golden hamsters, a serial sacrifice study. Cancer Res. 38: 1135-1143, 1978.

B226. Kennedy, A.R., Mondal, S., Heidelberger, C. and Little, J.B.: Enhancement of X-radiation transformation by a phorbol ester using C3H/10T½/C18 mouse embryo fibroblasts. Cancer Res. 38: 439-443, 1978.

B227. Kennedy, A.R., Worcester, J. and Little, J.B.: Deposition and localization of poponium-210 intratracheally instilled in the hamster lung as determined by autoradiography of freeze-dried sections. Radiat. Res. 69: 553-572, 1977.

B228. King, H.W.S., Osborne, M.R. and Brookes, P.: The identification of 3-methylcholanthrene-9,10-dihydrodiol as an intermediate in the binding of 3-methylcholanthrene to DNA in cells in culture. Chem. Biol. Interact. (In Press).

B229. King, H.W.S., Osborne, M.R. and Brookes, P.: The metabolism and DNA binding of 3-methylcholanthrene. Int. J. Cancer 20: 564-571, 1977.

B230. Kobbe, B., Cushman, M., Wogan, G.N. and Demain, A.L.: Production and antibacterial activity of malformin C, a toxic metabolite of Aspergillus Niger. Appl. Environ. Microbiol. 33: 996-997, 1977.

B231. Kojima, A., Tanaka-Kojima, Y. and Nishizuka, Y.: Experimental induction of autoimmunity in nude mice. In Nomura, T., Ohsawa, N., Tamaoki, N. and Fujiwara, K. (Eds.): Proceeding of the Second International Workshop on Nude Mice. Tokyo, University of Tokyo Press, 1977, pp. 127-137.

B232. Kopelovich, L., Conlon, S. and Pollack, R.: Defective organization of actin in cultured skin fibroblasts from patients with inherited adenocarcinoma. Proc. Natl. Acad. Sci. USA 74: 3019-3022, 1977.

B233. Kopelovich, L. and Wolfe, G.: Modification of ribonucleic acid by vitamin B_6. Specific interaction of pyridoxal 5'-phosphate with transfer ribonucleic acid. Biochemistry 16: 3721-3726, 1977.

B234. Koren, R., Shohami, E., Bibi, O. and Stein, W.D.: Uridine transport properties of mammalian cell membranes are not involved with growth control or oncogenesis. FEBS Lett. 86: 71-75, 1978.

B235. Koren, R., Shohami, E. and Yeroushalmi, S.: A kinetic analysis of the uptake of cytosine-β-D-arabinoside by rat-B77 cells. Differentiation between transport and phosphorylation. Biochem. Pharmacol. (In Press).

B236. Kouri, R.E., Imblum, R.L. and Prough, R.A.: Measurement of aryl hydrocarbon hydroxylase and NADH-dependent cytochrome c reductase activities in mitogen-activated human lymphocytes. In Nieburgs, H. (Ed.): Proceedings of the Third International Symposium on Detection and Prevention of Cancer. (In Press).

B237. Kouri, R.E., Imblum, R.L. and Prough, R.A.: Relationship between levels of aryl hydrocarbon hydroxylase activity and susceptibility to 3-methylcholanthrene and benzo(a)pyrene-induced cancers in inbred strains of mice. In Nieburgs, H. (Ed.): Proceedings of Third International Symposium on Detection and Prevention of Cancer. (In Press).

B238. Kozak, C.A., Lawrence, J.B. and Ruddle, F.H.: A sequential staining technique for the chromosomal analysis of interspecific mouse/hamster and mouse/human somatic cell hydrids. Exp. Cell Res. 105: 109-117, 1977.

B239. Kozak, C.A. and Ruddle, F.H.: Sexual and parasexual approaches to the genetic analysis of the laboratory mouse, Mus Musculus. In Vitro 12: 720-725, 1976.

B240. Kundu, A.B., Telang, N.T. and Banerjee, M.R.: The binding of 7,12-dimethylbenz(a)anthracene to mammary gland DNA in organ culture. J. Natl. Cancer Inst. (In Press).

B241. Kupper, R., Nagel, D., Gingell, R. and Brunk, G.: Synthesis of ^{14}C-labeled N-nitrosobis(2-hydroxypropyl)amine. J. Labeled Compd. (In Press).

B242. Lake, R.S., Freeman, A.E., Igel, H.J., Pezzutti, M.R. and Kropko, M.L.: In vitro culture of diploid human skin epithelial cells for chemical transformation studies. In Saffiotti, U. and Autrup, H. (Eds.): In Vitro Carcinogenesis Guide to the Literature, Recent Advances and Laboratory Procedures. Carcinogenesis Program, DCCP, NCI: Technical Report Series No. 44. U.S. DHEW, PHS, NIH, NCI. DHEW Publ. No. (NIH) 78-844. Wash.,D.C., U.S. Govt. Print. Off., 1978, pp. 283-291.

B243. Lam, L.K.T. and Farhat, K.: The use of trimethyl actyl chloride in the selective synthesis of 3-tert-butyl-4-methoxy-phenol - a BHA isomer. Org. Prep. Proced. Int. (In Press).

B244. Lane, B.P.: In vitro studies. In Harris, C.C.(Ed.): Pathogenesis and Therapy of Lung Cancer. (In Press).

B245. Lane, B.P.: In vitro carcinogenesis in tracheal organ culture. In Saffiotti, U. and Autrup, H. (Eds.): In Vitro Carcinogenesis Guide to the Literature, Recent Advances and Laboratory Procedures. Carcinogenesis Program, DCCP, NCI: Technical Report Series No. 44. U.S. DHEW, PHS, NIH, NCI. DHEW Publ. No. (NIH) 78-844. Wash.,D.C., U.S. Govt. Print. Off., 1978, pp. 123-131.

B246. Lane, B.P. and Mass, M.J.: Carcinogenicity and cocarcinogenicity of chromium carbonyl in heterotopic tracheal grafts. Cancer Res. 37: 1476-1479, 1977.

B247. Langenbach, R. and Kennedy, S.: Gangliosides and their cell density--dependent changes in control and chemically transformed C3H/10T1/2 cells. Exp. Cell Res. (In Press).

B248. Langenbach, R., Malick, L. and Kennedy, S.: Ganglioside and morphological changes in mouse embryo cells with time. Cancer Lett. 4: 13-19, 1977.

B249. Laskin, S. and Goldstein, B.D. (Eds.): Benzene Toxicity: A Critical Evaluation. J. Toxicol. Environ. Health (Supplement 2), 1977, 157 pp.

B250. Lavi, S. and Groner, Y.: The 5'-leader sequence and coding region of late SV40 mRNAs are derived from non-contiguous segments of the viral genome. Proc. Natl. Acad. Sci. USA 74: 5323-5327, 1977.

B251. Leavitt, J.C., Crawford, B.D., Barrett, J.C. and Ts'o, P.O.P.: Regulation of requirements for anchorage-independent growth of Syrian hamster fibroblasts by somatic mutation. Nature 269: 63-65, 1977.

B252. Lechner, J.F., Narayan, K.S., Ohnuki, Y., Babcock, M.S., Jones, L.W. and Kaighn, M.E.: Replicative epithelial cell cultures from normal human prostate. J. Natl. Cancer Inst. (In Press).

B253. Lee, K., Gold, B. and Mirvish, S.S.: Mutagenicity of 22 N-nitrosamides and related compounds for salmonella typhimurium TA1535. Mutat. Res. 48: 131-138, 1977.

B254. Lehninger, A.L.: Living systems as energy converters: energy in biological molecules. In Buvet, R., Allen, M.J. and Massue, J.-P. (Eds.): Living Systems as Energy Converters. Amsterdam, Elsevier/North Holland Biomedical Press, 1977, pp. 103-111.

B255. Lehninger, A.L.: Mitochondria and biological mineralization processes: an exploration. In Quagliariello, E., Palmieri, F. and Singer, T. (Eds.): Horizons in Biochemistry and Biophysics. Reading, Addison-Wesley, Vol. 4, 1977, pp. 1-30.

B256. Lehninger, A.L.: Proton transport and charge separation across the mitochondrial membrane coupled to electron flow. In Laszlo, P. (Ed.): Protons and Ions Involved in Fast Dynamic Phenomena. (In Press).

B257. Lehninger, A.L., Reynafarje, B. and Alexandra, A.: Stoichiometry of proton movements coupled to mitochondrial electron transport and ATP hydrolysis. In van Dam, K. and van Gelder, B.F. (Eds.): Structure and Function of Energy-Transducing Membranes. Amsterdam, Elsevier/North Holland Biomedical Press, 1977, pp. 95-106.

B258. Lehninger, A.L., Reynafarje, B., Vercesi, A. and Tew, W.P.: Characteristics of energy-dependent calcium influx and efflux systems of mitochondria. In Azzone, G.F., Avron, M., Metcalfe, J.C., Quagliariello, E. and Siliprandi, N. (Eds.): The Proton and Calcium Pumps. Amsterdam, Elsevier/North Holland Biomedical Press, 1978, pp. 203-214.

B259. Lehninger, A.L., Reynafarje, B., Vercesi, A. and Tew, W.P.: Transport and accumulation of Ca^{2+} in mitochondria. Ann. N.Y. Acad. Sci. (In Press).

B260. Lehninger, A.L., Vercesi, A. and Bababunmi, E.A.: Regulation of Ca^{2+} release from mitochondria by the oxidation-reduction state of pyridine nucleotides. Proc. Natl. Acad. Sci. (In Press).

B261. Leinwand, L.A., Fournier, R.E.K., Nichols, E.A. and Ruddle, F.H.: Assignment of the gene for adenosine kinase to chromosome 14 in Mus musculus by somatic cell hybridization. Cytogenet. Cell Genet. (In Press).

B262. Leinwand, L.A., Kozak, C.A. and Ruddle, F.H.: Assignment of the genes for triose phosphate isomerase to chromosome 6 and tripeptidase-1 to chromosome 10 in Mus musculus by somatic cell hybridization. Som. Cell Genet. (In Press).

B263. Leinwand, L.A., Nichols, E. and Ruddle, F.H.: Assignment of the gene for glyoxylase I to mouse chromosome 17 by somatic cell genetics. Biochem. Genet. (In Press).

B264. Leinwand, L.A. and Ruddle, F.H.: Assignment of the gene for dipeptidase-2 to Mus musculus chromosome 18 by somatic cell hybridization. Biochem. Genet. (In Press).

B265. Lijinsky, W.: Chemical structure and carcinogenesis of nitrosamines. In Proceedings of FDA Symposium on Structural Correlates of Carcinogenesis and Mutagenesis. (In Press).

B266. Lijinsky, W.: Chemicals can cause cancer. Life and Health 93: 34-36, 1978.

B267. Lijinsky, W.: Chronic toxicity tests in animals as predictors of carcinogenesis in man. Chem. Eng. News 55: 25-33 & 45-46.

B268. Lijinsky, W.: Nitrosamines and nitrosamides in the etiology of gastrointestinal cancer. Cancer 40: 2446-2449, 1977.

B269. Lijinsky, W.: Possible formation of N-nitroso compounds from amines and nitrite. In Proceedings of Second Symposium on Nitrite in Meat Products. Zeist, 1976, pp. 269-274.

B270. Lijinsky, W.: Standard setting for nitrites and nitrosamines. In Hiatt, H.H., Watson, J.D. and Winsten, J.A. (Eds.): Origins of Human Cancer. New York, Cold Spring Harbor Laboratory, Vol. 4, 1977, pp. 1807-1811.

B271. Lijinsky, W. and Taylor, H.W.: Nitrosamines and their precursors in food. In Hiatt, H.H., Watson, J.D. and Winsten, J.A. (Eds.): Origins of Human Cancer. New York, Cold Spring Harbor Laboratory, Vol. 4, 1977, pp. 1579-1590.

B272. Lijinsky, W. and Taylor, H.W.: Relative carcinogenic effectiveness of derivatives of diethylnitrosamine in rats. Cancer Res. (In Press).

B273. Lijinsky, W. and Taylor, H.W.: Transplacental chronic toxicity test of carbaryl with nitrite in rats. Food Cosmet. Toxicol. 15: 229-232, 1977.

B274. Lin, J-C., Hiasa, Y. and Farber, E.: Preneoplastic antigen as a marker for endoplasmic reticulum of putative premalignant hepatocytes during liver carcinogenesis. Cancer Res. 37: 1972-1981, 1977.

B275. Ling, V.: A membrane-altered mutant cold-sensitive for growth. J. Cell Physiol. 91: 209-224, 1977.

B276. Ling, V.: Genetic aspects of frug resistance in somatic cells. In E. Schabel (Ed.): Antibiotics and Chemotherapy. Basel, S. Karger, Vol. 23, 1977, pp. 191-199.

B277. Ling, V. and Baker, R.M.: Dominance of the colchicine-resistant phenotype in hybrid CHO cells. Som. Cell Genet. (In Press).

B278. Ling, V., Carlsen, S.A. and See, Y.P.: Altered drug permeability in mammalian cell mutants. In Miller, M.W. and Shamoo, A. (Eds.): Advances in Experimental Medicine and Biology. New York, Plenum Press, Vol. 84, 1977, pp. 247-264.

B279. Lipkin, M.: Growth kinetics of normal and premalignant gastrointestinal epithelium. In Drewinko, B. and Humphrey, R. (Eds).: M. D. Anderson 29th Annual Symposium on Fundamental Cancer Research, Growth Kinetics and Biochemical Regulation of Normal and Malignant Cells. Baltimore, Williams and Wilkins Co., 1977, pp. 569-589.

B280. Lipkin, M.: Identification of susceptibility to gastrointestinal neoplasia. Acta Endoscopica et Radiocinematographica. (In Press).

B281. Lipkin, M.: Identifying susceptibility of human population groups to colon cancer. Adv. Cancer Res. (In Press).

B282. Lipkin, M.: Neoplastic transformation of cells in the gastrointestinal tract of man and rodent. In Farber, E., Kawachi, T., Sugano, H., Sugimura, T. and Weisburger, J.H. (Eds.): Proceedings of 7th International Symposium of the Princess Takamatsu Research Fund; Pathophysiology of Carcinogenesis in Digestive Organs. Tokyo, University of Tokyo Press, 1977, pp. 413-428.

B283. Lipkin, M.: Proliferative abnormalities in gastrointestinal cells and the development of neoplasia. Eur. J. Cancer (In Press).

B284. Lipkin, M. and Deschner, E.E.: Gastrointestinal neoplasia: An investigative approach. In Mulvihill, J.J., Miller, R.W. and Fraumeni, J.F.,Jr., (Eds.): Genetics of Human Cancer. New York, Raven Press, Vol. 3, 1977, pp. 369-375.

B285. Lipkin, M., Sherlock, P. and Winawer, S.J.: Early diagnosis and detection of colorectal cancer in high risk population groups. In Lipkin, M. and Good, R.A. (Eds.): Gastrointestinal Tract Cancer. (In Press).

B286. Little, J.B. and Kennedy, A.R.: Evaluation of alpha radiation-induced respiratory carcinogenesis in Syrian hamsters: Total dose and dose-rate. Prog. Exp. Tumor Res. (In Press).

B287. Little, J.B., Kennedy, A.R. and McGandy, R.B.: Effect of dose distribution on the induction of experimental lung cancer by alpha radiation. Health Phys. (In Press).

B288. Little, J.B., McGandy, R.B. and Kennedy, A.R.: Interactions between polonium-210 alpha radiation, benzo(a)pyrene and saline instillations in the induction of experimental lung cancer. Cancer Res. (In Press).

B289. Longnecker, D.S., French, J., Hyde, E., Lilja, H.S. and Yager, J.D., Jr.: Effect of age on nodule induction by azaserine and DNA synthesis in rat pancreas. J. Natl. Cancer Inst. 58: 1769-1775, 1977.

B290. Love, L.A., Pelfrene, A. and Garcia, H.: Acinar adenomas of the pancreas in MRC-wistar rats. J. Comp. Pathol. 87: 307-310, 1977.

B291. Love, L.A., Pelfrene, A. and Garcia, H.: Effect of intestinal microflora suppression on liver carcinogenicity of dimethylnitrosamine in Syrian hamsters: Brief communication. J. Natl. Cancer Inst. 58: 1835-1836, 1977.

B292. Lyle, R.E., Fribush, H.M., Lyle, G.G. and Saavedra, J.E.: Regiospecificity and conformational specificity in oxime alkylation of a geometrical enantiomeric isomer. J. Org. Chem. 43: 1275-1280, 1978.

B293. Lyle, R.E., Lyle, G.G., Fribush, H.M. and Saavedra, J.E.: Electronic properties of the nitrosamine function. In Walker, E. and Lyle, R.E. (Eds.): Proceedings of the 5th IARC Workshop on Nitrosamines. IARC Scientific Publications. (In Press).

B294. Lyle, R.E., Saavedra, J.E. and Coppola, B.P.: The preparation of the monoaroylpiperazine, 1-benzoylpiperazine. Org. Prep. Proc. Int. (In Press).

B295. Mahboubi, E.: The epidemiology of oral cavity, pharyngeal and esophageal cancer outside of North America and Western Europe. Cancer 40: 1879-1886, 1977.

B296. Mahboubi, E., Day, N.E., Ghadirian, P. and Salmasizadeh, S.: Negligible role of alcohol and tobacco in the etiology of esophageal cancer in Iran -- A case control study. Detect. Prevent. Cancer 4: 1149-1159, 1977.

B297. Mahboubi, E. and Shubik, P.: Epidemiological relationship between steroid hormones and liver lesions. J. Toxicol. Environ. Health 3: 208-218, 1977.

B298. Malick, L. and Toth, B.: Injection replication of the vasculature of angiosarcomas in liver of Syrian golden hamster. In Proceedings of Workshop on Biomedical Applications. Scanning Electron Microscopy. Chicago, IIT Research Institute, 1977.

B299. Maloney, J.R. and Lyle, R.E.: Oxidative deoximation with pyridinium chlorochromate. Synthesis (In Press).

B300. Mangino, M.M., Lijinsky, W. and Singer, G.M.: Synthesis of deuterium labeled nitrosomethylethyamine. J. Labeled Compd. (In Press).

B301. Manning, W.B., Tomaszewski, J.E., Muschik, G.M. and Sato, R.: A general synthesis of 1-, 2-, 3-, and 4-substituted benz(a)anthracene-7,12-diones. J. Org. Chem. 3465-3468, 1977.

B302. Marchok, A.C., Rhoten, J.C., Griesemer, R.A. and Nettesheim, P.: Increased in vitro growth capacity of tracheal epithelium exposed in vivo to 7,12-dimethylbenz(a)anthracene. Cancer Res. 37: 1811-1821, 1977.

B303. Marchok, A.C., Rhoton, J.C. and Nettesheim, P.: In vitro development of oncogenicity in cell lines established from tracheal epithelium pre-exposed in vivo to 7,12-dimethylbenz(a)anthracene. Cancer Res. (In Press).

B304. Marchok, A.C. and Terzaghi, M.: In vivo-in vitro studies with respiratory tract epithelium exposed to carcinogen. In Saffiotti, U. and Autrup, H. (Eds.): In Vitro Carcinogenesis Guide to the Literature, Recent Advances and Laboratory Procedures. Carcinogenesis Program, DCCP, NCI: Technical Report Series No. 44. U.S. DHEW, PHS, NIH, NCI. DHEW Publ. No. (NIH) 78-844. Wash.,D.C., U.S. Govt. Print. Off., 1978, pp. 132-138.

B305. Mark-Malchoff, D., Marinetti, G.V., Hare, J.D. and Meisler, A.I.: Characterization of phosphatidylthreonine in polyoma virus transformed fibroblasts. Biochem. (In Press).

B306. Mark-Malchoff, D., Marinetti, G.V., Hare, J.D. and Meisler, A.I.: Elevation of a threonine phospholipid in polyoma virus transformed hamster embryo fibroblasts. Biochem. Biophys. Res. Commun. 75: 589-593, 1977.

B307. Marsh, S. and Grandjean, C.: Combined ultraviolet absorbance and fluorescence monitoring: An aid to identification of polycyclic aromatic hydrocarbon metabolites by high-pressure liquid chromatography. J. Chromatogr. 147: 411-414, 1978.

B308. Maskens, A.P. and Deschner, E.E.: Tritiated thymidine incorporation into epithelial cells of normal-appearing colorectal mucosa of cancer patients. J. Natl. Cancer Inst. 58: 1221-1224, 1977.

B309. McCaw, B.A., Dipple, A., Young, S. and Roberts, J.J.: Excision of hydrocarbon-DNA adducts and consequent cell survival in normal and repair defective human cells. Chem. Biol. Interact. (In Press).

B310. McDowell, E.M., Becci, P.J., Barrett, L.A. and Trump, B.F.: Morphogenesis and classification of lung cancer. In Harris, C.C. (Ed.): Pathogenesis and Therapy of Lung Cancer. (In Press).

B311. McDowell, E.M., Becci, P.J. and Trump, B.F.: Histogenesis of epidermoid metaplasia, carcinoma in situ and carcinomas in the human lung; comparison with the hamster. Progress in Experimental Tumor Research. (In Press).

B312. Meadows, A.T., D'Angio, G.J., Evans, A.E., Jaffe, N., Schweisguth, O. and Van Eys, J.: Spontaneous and treatment-related second malignant neoplasms in children. In Severi, L. (Ed.): Proceedings of Tumors of Early Life in Man and Animals. (In Press).

B313. Meiss, H.K., Talavera, A. and Nishimoto, T.: A recurreing temperature-sensitive mutant class of BHK-21 cells. Som. Cell Genet. 4: 125-130, 1978.

B314. Miller, R.A. and Ruddle, F.H.: Properties of teratocarcinoma-thymus somatic cell hybrids. Som. Cell Genet. 3: 247-261, 1977.

B315. Miller, R.A. and Ruddle, F.H.: Teratocarcinoma x Friend erythroleukemia cell hybrids resemble their pluripotent embryonal carcinoma parent. Dev. Biol. 56: 157-173, 1977.

B316. Mirvish, S.S.: N-nitroso compounds, nitrite and nitrate: Possible implications for the causation of human cancer. J. Water Res. 1977.

B317. Mirvish, S.S.: N-nitroso compounds: Their chemical and in vivo formation and possible importance as environmental carcinogens. J. Toxicol. Environ. Health. (In Press).

B318. Mirvish, S.S., Karlowski, K., Sams, J.P. and Arnold, S.D.: Studies related to nitrosamine formation: Nitrosation in solvent: water and solvent systems, nitrosomethylurea formation in the rat stomach, and analysis of a fish product for ureas. IARC Scientific Publications. (In Press).

B319. Mirvish, S.S., Lee, K. and Gold, B.: Mutagenicity of 22 N-nitrosamides for Salmonella typhimurium TA 1535. Mutat. Res. 48: 131-138, 1977.

B320. Mirvish, S.S., Sams, J.P. and Hecht, S.S.: Kinetics of nornicotine and anabasine nitrosation in relation to N'-nitrosonornicotine occurrence in tobacco and to tobacco-induced cancer. J. Natl. Cancer Inst. (In Press).

B321. Montesano, R., Drevon, C., Kuroki, T., Saint Vincent, L., Handleman, S., Sanford, K.K., DeFoe, D. and Weinstein, I.B.: Tests for malignant transformation of liver cells in culture. Cytology, growth in soft agar and production of plasminogen activator. J. Natl. Cancer Inst. 59: 1651-1658, 1977.

B322. Moore, W.E.C.: Anaerobes as normal flora: Gastrointestinal tract. Excerpta Med. 222-228, 1977.

B323. Mori, T.: Ultrastructure of the uterine epithelium of mice treated neonatally with estrogen. Acta Anat. 99: 462-468, 1977.

B324. Mori, T., Komuro, M. and Nagasawa, H.: Effects of neonatal injections of anti-estrogen(MER-25) on ovaries and genital tracts of mice. IRCS J. Med. Sci. 5: 384-387, 1977.

B325. Mori, T. and Nishizuka, M.: Additional effects of postpubertal estrogen injections on the vaginal epithelium in neonatally estrogenized mice. Acta Anat. 100: 369-374, 1978.

B326. Moschel, R.C., Baird, W.M. and Dipple, A.: Metabolic activation of the carcinogen 7,12-dimethylbenz(a)anthracene for DNA binding. Biochem. Biophys. Res. Commun. 76: 1092-1098, 1977.

B327. Moser, G.C. and Meiss, H.K.: A cytological procedure to screen mammalian temperature-sensitive mutants for cell-cycle-related defects. Som. Cell Genet. 3: 449-465, 1970.

B328. Mossman, B.T.: Autoradiography for determination of DNA synthesis in hamster bladder epithelium. Tissue Culture Association Manual 3: 663-665, 1977.

B329. Mossman, B.T., Adler, K.B. and Craighead, J.E.: The interaction of carbon particles with tracheal epithelium in organ culture. Environ. Res. (In Press).

B330. Mossman, B.T. and Craighead, J.E.: Use of hamster tracheal organ cultures for assessing the cocarcinogenic effects of inorganic particulates on the respiratory epithelium. Prog. Exp. Tumor Res. (In Press).

B331. Mossman, B.T. and Craighead, J.E.: Organ culture of the hamster bladder epithelium. Tissue Culture Association Manual 3: 623-624, 1977.

B332. Mossman, B.T. and Craighead, J.E.: Induction of tumors in hamster tracheal grafts with 3-methylcholanthrene coated Lycra fibers. Cancer Res. (In Press).

B333. Mossman, B.T., Heintz, N., MacPherson, B.V. and Craighead, J.E.: Squamous metaplasia of the tracheal epithelium in organ culture. II. Nutritional influences. Proc. Soc. Exp. Biol. Med. 157: 500-505, 1978.

B334. Mossman, B.T., Kessler, J.B., Ley, B.W. and Craighead, J.E.: Interaction of crocidolite asbestos with hamster respiratory mucosa in organ culture. Lab. Invest. 36: 131-139, 1977.

B335. Nagel, D.L., Kupper, R., Antonsen, K. and Wallcave, L.: Synthesis of alkyl-substituted benzo(c)phenanthrenes and chrysenes by photocyclization. J. Org. Chem. 42: 3626-3628, 1977.

B336. Nagel, D.L., Wallcave, L., Toth, B. and Kupper, R.: Formation of methylhydrazine from acetaldehyde N-methyl-N-formylhydrazone, a component of Gyromitra esculenta. Cancer Res. 37: 3458-3460, 1977.

B337. Nakanishi, K., Kasai, H., Cho, H., Harvey, R.G., Jeffrey, A.M., Jennette, K.W. and Weinstein, I.B.: Absolute configuration of an RNA adduct formed in vivo by metabolism of benzo(a)pyrene. J. Am. Chem. Soc. 99: 258-260, 1977.

B338. Narisawa, T., Reddy, B.S. and Weisburger, J.H.: Effect of bile acids and dietary fat on large bowel carcinogenesis in animal model. Gastroenterol. Jpn. (In Press).

B339. Nechaev, A., Halberg, F., Mittelman, A. and Tritsch, G.L.: Circannual variation in human erythrocyte adenosine aminohydrolase. Chronobiologia 4: 191-198, 1977.

B340. Nettesheim, P. and Griesemer, R.A.: Experimental models for studies of respiratory tract carcinogenesis. In Harris, C.C. (Ed.): Pathogenesis and Therapy of Lung Cancer. (In Press).

B341. Nettesheim, P., Griesemer, R.A., Martin, D.H. and Caton, J.E., Jr.: Induction of preneoplastic and neoplastic lesions in grafted rat tracheas continuously exposed to benzo(a)pyrene. Cancer Res. 37: 1272-1278, 1977.

B342. Nettesheim, P., Marchok, A.C. and Terzaghi, M.: In vivo-in vitro studies of epithelial neoplasia. In Ts'o, P.O.P. and Gelboin, H.V. (Eds.): Polycyclic Hydrocarbons and Cancer: Chemistry, Molecular Biology and Environment. (In Press).

B343. Newbold, R.F., Wigley, C.B., Thompson, M.H. and Brookes, P.: Cell-mediated mutagenesis in cultured Chinese hamster cells by carcinogenic polycyclic hydrocarbons: Nature and extent of the associated hydrocarbons-DNA reaction. Mutat. Res. 43: 101-116, 1977.

B344. Nishimoto, T. and Basilico, C.: Analysis of a method for selecting temperature sensitive mutants of BHK cells. Som. Cell Genet. (In Press).

B345. Nishizuka, Y., Sakakura, T. and Taguchi, O.: Mechanism of ovarian tumorigenesis in mice after neonatal thymectomy. In Rice, J.M., Bern, H.A. and Mohr, U. (Eds.): Perinatal Carcinogenesis. Natl. Cancer Inst. Monogr. (In Press).

B346. Oren, M., Lavi, S. and Winocour, E.: The structure of a cloned substituted SV40 genome. Virology. (In Press).

B347. Osborne, M.R., Harvey, R.G. and Brookes, P.: The reaction of trans-7,8-dihydroxy-anti-9,10-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene with DNA involves attack at the N^7-position of guanine moieties. Chem. Biol. Interact. 20: 123-130, 1978.

B348. Owyang, C., Dozois, R.R., DiMagno, E.P. and Go, V.L.W.: Relationships between fasting and postprandial pancreatico-duodenal pressures, pancreatic secretion, and duodenal volume flow in the dog. Gastroenterology 73: 1046-1049, 1977.

B349. Paigen, B., Gurtoo, H.L., Minowada, J., Houten, L., Vincent, R., Paigen, K., Parker, N.B., Ward, E. and Hayner, N.T.: Questionable relationship of aryl hydrocarbon hydroxylase to lung cancer risk. N. Engl. J. Med. 297: 346-350, 1977.

B350. Paigen, B., Gurtoo, H.L., Minowada, J. and Paigen, K.: Aryl hydrocarbon hydroxylase in cultured human lymphocytes. In Ullrich, V., Roots, I., Hildebrandt, A., Estabrook R. and Conney, A. (Eds.): Microsomes by Drug Oxidation. London, Pergamon Press, 1977, pp. 418-425.

B351. Paigen, B., Gurtoo, H.L., Minowada, J., Ward, E., Houten, L., Paigen, K., Reilly, A. and Vincent, R.: Genetics of aryl hydrocarbon hydroxylase in the human population. In Ts'o, P.O.P. and Gelboin, H.V. (Eds.): Polycyclic Hydrocarbons and Cancer: Chemistry, Molecular Biology and Environment. (In Press).

B352. Paigen, B., Gurtoo, H.L., Ward, E., Minowada, J., Houten, L., Vincent, R., Parker, N.B. and Vaught, J.: Human aryl hydrocarbon hydroxylase and cancer risk. In Jones, P.W. and Freudenthal, R.J. (Eds.): Carcinogenesis, Vol. 3: Polynuclear Aromatic Hydrocarbons. (In Press).

B353. Paigen, B., Minowada, J., Gurtoo, H.L., Paigen, K., Parker, N.B., Ward, E., Hayner, N.T., Bross, I.D.J., Boch, F. and Vincent, R.: Distribution of aryl hydrocarbon hydroxylase inducibility in human lymphocytes. Cancer Res. 37: 1829-1837, 1977.

B354. Palmer, W.G., Allen, T.J. and Tomaszewski, J.E.: Metabolism of 7,12-dimethylbenz(a)anthracene by macrophages and uptake of macrophage-derived metabolites by respiratory tissues in vitro. Cancer Res. 38: 1087-1092, 1978.

B355. Palmer, W.G. and Ulland, B.M.: Serum haptoglobin levels in mice with 7,12-dimethylbenz(a)anthracene-induced tumors. Oncology. (In Press).

B356. Parker, J., Pollard, J., Friesen, J.D. and Stanners, C.P.: Stuttering: High level mistranslation in animal and bacterial cells. Proc. Natl. Acad. Sci. USA. (In Press).

B357. Peisach, J.: Magnetic probes of metal binding sites in copper proteins. In Singer, T.P. and Ondarza, R.N. (Eds.): Mechanisms of Oxidizing Enzymes. New York, Elsevier North Holland, 1978, pp. 285-306.

B358. Pelfrene, A.: Early vascular modifications induced by asbestos fibers in the hamster cheek pouch. Microvasc. Res. 13: 261-265, 1977.

B359. Pfeffer, L.M. and Kopelovich, L.: Differential genetic susceptibility of cultured human skin fibroblasts to transformation by Kirsten murine sarcoma virus. Cell 10: 313-320, 1977.

B360. Pienta, R.J., Shah, M.J., Lebherz, W.B., III and Andrews, A.W.: Correlation of bacterial mutagenicity and hamster cell transformation induced by 2,4-toluenediamine. Cancer Lett. 3: 45-52, 1977.

B361. Pietropaolo, C., Yamaguchi, N., Weinstein, I.B. and Glick, M.C.: Glycopeptides from normal and transformed epithelial cells: Studies on mutants temperature sensitive for the transformation phenotype. Int. J. Cancer 20: 738-747, 1977.

B362. Pour, P.: Islet cells as a component of pancreatic ductal neoplasms. Am. J. Pathol. 90: 295-316, 1978.

B363. Pour, P.: The unique role of the hamster in specific carcinogenesis studies. Prog. Exp. Tumor Res. (In Press).

B364. Pour, P.: Tumors of the skeleton. In Manual on the Pathology of Tumors in Laboratory Animals. IARC Scientific Publications. (In Press).

B365. Pour, P., Althoff, J., Kruger, F.W. and Mohr, U.: A potent pancreatic carcinogen in Syrian hamsters: N-nitrosobis(2-oxopropyl)amine. J. Natl. Cancer Inst. 58: 1449-1453, 1977.

B366. Pour, P., Althoff, J., Kruger, F.W. and Mohr, U.: Improvement of pancreatic cancer model by modified treatment with N-nitrosobis(2-oxopropyl)-amine. Cancer Lett. 2: 233-238, 1977.

B367. Pour, P., Althoff, J., Kruger, F.W. and Mohr, U.: The effect of N-nitrosobis(2-oxopropyl)amine after oral administration to hamsters. Cancer Lett. 2: 323-326, 1977.

B368. Pour, P., Althoff, J. and Nagel, D.: Induction of epithelial neoplasms by local application of N-nitrosobis(2-hydroxypropyl)amine and N-nitrosobis-(2-acetoxypropyl)amine. Cancer Lett. 3: 109-113, 1977.

B369. Pour, P., Althoff, J. and Takahashi, M.: Early lesions of pancreatic ductal carcinoma in the hamster model. Am. J. Pathol. 88: 291-303, 1977.

B370. Pour, P. and Birt, D.: Spontaneous diseases--Their implications in toxicological research: facts, thoughts, and suggestions. Prog. Exp. Tumor Res. (In Press).

B371. Pour, P., Ii, Y. and Althoff, J.: Comparative studies on spontaneous tumor incidence based on systematic histologic examination of one rat and two hamster strains from the same colony. Prog. Exp. Tumor Res. (In Press).

B372. Pour, P., Stanton, M.F., Kuschner, M., Laskin, S. and Shabad, L.M.: Tumours of the respiratory tract. In Pathology of Tumours in Laboratory Animals. Lyon, International Agency for Research on Cancer, 1976, Vol. 1, pp. 1-40.

B373. Prough, R.A. and Dunn, D.L.: The application of HPLC techniques to monitor the oxidative metabolism of carcinogens and drugs. In Hawk, G.L. (Ed.): Liquid Chromatography Symposium 1, Biological/Biomedical Applications of Liquid Chromatography. (In Press).

B374. Prough, R.A., Sipal, Z. and Jakobsson, S.W.: Metabolism of benzo(a)-pyrene by human lung microsomal fractions. Life Sci. 21: 1629-1636, 1977.

B375. Putman, D.L., Park, D.K., Rhim, J.S., Steuer, A.F. and Ting, R.C.: Correlation of cellular aggregation of transformed cells with their growth in soft agar and tumorigenic potential. Proc. Soc. Exp. Biol. Med. 155: 487-494, 1977.

B376. Quimby, F.W., Gebert, R., Syamal, D., Andre-Schwartz, J., Tannenberg, W.J., Lewis, R.M., Weinstein, I.B. and Schwartz, R.S.: Characterization of a retrovirus that cross-reacts serologically with canine and human SLE. Clin. Immunol. Immnopathol. 9: 194-210, 1978.

B377. Rao, M.S. and Reddy, J.K.: Pathology of tumors developed in guinea pigs given intraperitoneal injections of N-methyl-N-nitrosourea. Neoplasma 24: 57-62, 1977.

B378. Rao, M.S. and Reddy, J.K.: Sub-cellular changes in exocrine pancreas of the guinea pig induced by azaserine. Biochem. Exp. Biol. 12: 175-183, 1976.

B379. Rasco, M.A., Jacobs, M.M. and Griffin, A.C.: Effects of selenium on aryl hydrocarbon hydroxylase activity in cultured human lymphocytes. Cancer Lett. 3: 295-301, 1977.

B380. Reddy, B.S.: Carcinogenesis of the colon and rectum. In Enker, W.E. (Ed.): Carcinoma of the Colon and Rectum. (In Press).

B381. Reddy, B.S., Hedges, A., Laakso, J. and Wynder, E.L.: Fecal constituents of a high-risk North American and a low-risk Finnish population for the development of colon cancer. Cancer Lett. (In Press).

B382. Reddy, B.S., Hedges, A., Laakso, K. and Wynder, E.L.: Metabolic epidemiology of large bowel cancer: Fecal bulk and constituents of a high-risk North American and a low-risk Finnish population. Cancer (In Press).

B383. Reddy, B.S., Mangat, S., Sheinfil, A., Weisburger, J.H. and Wynder, E.L.: Effect of type and amount of dietary fat and 1,2-dimethylhydrazine on biliary bile acids, fecal bile acids, and neutral sterols in rats. Cancer Res. 37: 2132-2137, 1977.

B384. Reddy, B.S., Mangat, S., Weisburger, J.H. and Wynder, E.L.: Effect of high-risk diets for colon carcinogenesis on intestinal mucosal and bacterial β-glucuronidase activity in F344 rats. Cancer Res. 37: 3533-3536, 1977.

B385. Reddy, B.S., Mastromarino, A. and Wynder, E.L.: Diet and metabolism: Large bowel cancer. Cancer 39: 1815-1819, 1977.

B386. Reddy, B.S., Watanabe, K. and Weisburger, J.H.: Effect of high-fat diet on colon carcinogenesis in F344 rats treated with 1,2-dimethylhydrazine, methylazoxymethanol acetate or methylnitrosourea. Cancer Res. 37: 4156-4159, 1977.

B387. Reddy, B.S., Watanabe, K., Weisburger, J.H. and Wynder, E.L.: Promoting effect of bile acids in colon carcinogenesis in germfree and conventional rats. Cancer Res. 37: 3238-3242, 1977.

B388. Reddy, B.S., Weisburger, J.H. and Wynder, E.L.: Colon Cancer: Bile salts as tumor promoters. In Slaga, T.J., Sivak, A. and Boutwell, R.K. (Eds.): Carcinogenesis, Vol. 2. Mechanisms of Tumor Promotion and Carcinogenesis. New York, Raven Press, 1978, pp. 453-464.

B389. Regan, J.D.: Dye-light therapy for herpes simplex lesions. Photochem. Photobiol. 25: 333, 1977.

B390. Regan, J.D.: Interaction of carcinogens with human cell DNA: Damage and repair. In Snyder, R.H. (Ed.): Active Intermediates: Formation, Toxicity and Inactivation. New York, Plenum Press, 1977, pp. 483-491.

B391. Regan, J.D.: Photobiology in medicine. In Proceedings of the VII International Congress on Photobiology. London, Plenum Press, 1977, pp. 387-390.

B392. Regan, J.D., Carrier, W.L., Smith, D.P. and Waters, R.: Pyrimidine dimer excision in human cells and skin cancer. Natl. Cancer Inst. Monogr. (In Press).

B393. Regan, J.D., Francis, A.A., Dunn, W.C., Hernandez, O., Yagi, J. and Jerina, D.M.: Repair of DNA damaged by mutagenic metabolites of benzo(a)-pyrene in human cells. Chem. Biol. Interact. (In Press).

B394. Regan, J.D. and Setlow, R.B.: The effect of proflavin plus visible light on the DNA of human cells. Photochem. Photobiol. 25: 345-346, 1977.

B395. Reynafarje, B., Brand, M.D., Alexandre, A. and Lehninger, A.L.: Determination of the H^+/site and Ca^{2+}/site ratios of mitochondrial electron transport. Methods Enzymol. (In Press).

B396. Reynafarje, B. and Lehninger, A.L.: Electric charge stoichiometry of calcium translocation in mitochondria. Biochem. Biophys. Res. Commun. 77: 1273-1279, 1977.

B397. Roebuck, B.D. and Longnecker, D.S.: Species and rat strain variation in pancreatic nodule induction by azaserine. J. Natl. Cancer Inst. 59: 1273-1277, 1977.

B398. Roebuck, B.D., Siegel, W.G. and Wogan, G.N.: In vitro metabolism of aflatoxin B_2 by animal and human liver. Cancer Res. 38: 999-1002, 1978.

B399. Roebuck B.D. and Wogan, G.N.: Species comparison of in vitro metabolism of aflatoxin B_1. Cancer Res. 37: 1649-1656, 1977.

B400. Rogan, E. and Cavalieri, E.: Differences between nuclear and microsomal cytochrome P-450 in uninduced and induced rat liver. Mol. Pharmacol. (In Press).

B401. Rogan, E., Roth, R. and Cavalieri, E.: Enzymology of polycyclic hydro-carbon binding to nucleic acids. In Proceedings of the 2nd International Symposium on Polynuclear Aromatic Hydrocarbons. (In Press).

B402. Rogan, E., Roth, R., Katomski, P., Benderson, J. and Cavalieri, E.: Binding of benzo(a)pyrene at the 1,3,6 positions to nucleic acids in vivo with rat liver microsomes and nuclei. Chem. Biol. Interact. (In Press).

B403. Rogers, A.E.: Dietary effects on carcinogenicity of drugs and related compounds. In Hathcock, J. (Ed.): Nutrition and Drug Interrelations. (In Press).

B404. Rogers, A.E.: Effects of dietary factors on hepatocarcinogenesis in rats. In Newberne, P.M. and Butler, W. (Eds.): Proceedings of Conference on Hepatoma in Rats. (In Press).

B405. Rosenkranz, H.S., McCoy, E.C., Anders, M., Speck, W.T. and Bickers, D.: The use of microbial assay systems in the detection of environmental mutagens in complex mixtures. In Symposium on Application of Short-Term Bioassays in the Fractionation of Analysis of Complex Environmental Mixtures. (In Press).

B406. Rosenkranz, H.S., McCoy, E.C., Biuso, L. and Speck, W.T.: Short-term microbial assays in the assessment of carcinogenic risk. ATLA Symposium. (In Press).

B407. Rosiello, A.P., Essigmann, J.M. and Wogan, G.N.: Rapid and accurate determination of the median lethal dose (LD_{50}) and its error using a small computer. J. Toxicol. Environ. Health 3: 797-809, 1977.

B408. Ross, A.E. and Mirvish, S.S.: Metabolism of N-nitrosohexamethyleneimine to give 1,6-hexanediol bound to rat liver nucleic acids. J. Natl. Cancer Inst. 58: 651-655, 1977.

B409. Rozengurt, E., Stein, W.D. and Wigglesworth, M.: Uptake of nucleosides in density-inhibited cultures of 3T3 cells. Nature 267: 442-447, 1977.

B410. Rustia, M.: The role of hormone imbalance in transplacental carcinogenesis induced in Syrian hamsters by sex hormones. Natl. Cancer Inst. Monogr. (In Press).

B411. Sachs, L.: Control of cloning of normal human T lymphocytes by transferrin, albumin and different lectins. Clin. Exp. Immunol. (In Press).

B412. Sadzikowski, M.R., Sperry, J.F. and Wilkins, T.D.: A cholesterol-reducing bacterium from human feces. Appl. Environ. Microbiol. 34: 355-362, 1977.

B413. Salomon, R., Bar-Joseph, M., Soreq, H., Gozes, I. and Littauer, U.Z.: Translation in vitro of Carnation Mottle virus RNA: Regulatory function of the 3' region. Virology. (In Press).

B414. Salter, D.W., Custead-Jones, S. and Cook, J.S.: Quercetin inhibits hexose transport in a human diploid fibroblast. J. Membr. Biol. (In Press).

B415. Salyers, A.A., Harris, C.J. and Wilkins, T.D.: Breakdown of psyllium hydrocolloid by strains of Bacteroides ovatus from the human intestinal tract. Can. J. Microbiol. (In Press).

B416. Salyers, A.A., Palmer, J.K. and Wilkins, T.D.: Degradation of polysaccharides by intestinal bacterial enzymes. Am. J. Clin. Nutr. (In Press).

B417. Salyers, A.A., Palmer, J.K. and Wilkins, T.D.: Laminarinase (beta-glucanase) activity in Bacteroides from the human colon. Appl. Environ. Microbiol. 33: 1118-1124, 1977.

B418. Salyers, A.A., Sperry, J.F., Wilkins, T.D., Walker, A.R.P. and Richardson, N.J.: Neutral steroid concentrations in the feces of North American white and South African black populations at different risk for cancer of the colon. S. Afr. Med. J. 51: 823-827, 1977.

B419. Salyers, A.A., West, S.E.H., Vercellotti, J.R. and Wilkins, T.D.: Fermentation of mucins and plant polysaccharides by anerobic bacteria from the human colon. Appl. Environ. Microbiol. 34: 529-533, 1977.

B420. Sani, B.P. and Banerjee, C.K.: Purification and properties of retinoic acid-binding protein from chick embryo skin. Biochem. J. (In Press).

B421. Sani, B.P. and Titus, B.C.: Retinoic acid-binding protein in experimental tumors and in tissues with metastic tumor foci. Cancer Res. 37: 4031-4034, 1977.

B422. Sani, B.P., Titus, B.C. and Banerjee, C.K.: Quantitation of binding affinities of retinoids to retinoic acid-binding protein and serum albumin. Biochem. J. (In Press).

B423. Sansone, E.B. and Fox, J.G.: Potential chemical contamination in chronic animal feeding studies: Evaluation of gelled feed and wire- and solid-bottom caging systems. Lab. Anim. Sci. 27: 457-465, 1977.

B424. Sansone, E.B. and Lijinsky, W.: Carcinogens in schools. Science 196: 1272, 1977.

B425. Sansone, E.B. and Losikoff, A.M.: A note on the chemical contamination resulting from the transfer of solid and liquid materials in hoods. Amer. Ind. Hyg. Assoc. J. 38: 489-491, 1977.

B426. Sansone, E.B., Losikoff, A.M. and Pendleton, R.A.: Potential hazards from feeding test chemicals in carcinogen bioassay research. Toxicol. Appl. Pharmacol. 39: 435-450, 1977.

B427. Sansone, E.B., Losikoff, A.M. and Pendleton, R.A.: Sources and dissemination of contamination in material handling operations. Amer. Ind. Hyg. Assoc. J. 38: 433-442, 1977.

B428. Sansone, E.B. and Slein, M.W.: Application of the microbiological safety experience to work with chemical carcinogens. Amer. Ind. Hyg. Assoc. J. 37: 711-720, 1976.

B429. Sansone, E.B. and Slein, M.W.: Redispersion of indoor surface contamination: A review. J. Haz. Matr. (In Press).

B430. Sansone, E.B. and Tewari, Y.B.: The permeability of laboratory gloves to selected solvents. Amer. Ind. Hyg. Assoc. J. 39: 169-174, 1978.

B431. Sansone, E.B. and Tewari, Y.B.: Penetration of glove materials by 1, 2-dibromo-3-chloropropane, ethylene dibromide, and acrylonitrile. Amer. Ind. Hyg. Assoc. J. (In Press).

B432. Sansone, E.B. and Tewari, Y.B.: The permeability of laboratory gloves to selected nitrosamines. In Walker, E.A., Castegnaro, M., Griciute, L. and Lyle, R.E. (Eds.): Environmental Aspects of N-Nitroso Compounds. IARC Scientific Publications. (In Press).

B433. Sansone, E.B., Wolochow, H. and Chatigny, M.A.: Potential hazard associated with removal of needles from septa in injection ports of a gas chromatograph. Anal. Chem. 49: 670-671, 1977.

B434. Schmidt, M., Deschner, E.E., Thaler, T.H., Clements, L. and Good, R.A.: Gastrointestinal cancer studies in the human to nude mouse heterotransplant system. Gastroenterology 72: 829-837, 1977.

B435. Seeger, R.C., Rayner, S.A., Banerjee, A., Chung, H., Laug, W.E., Neustein, H.B. and Benedict, W.F.: Morphology, growth, chromosomal pattern, and fibrinolytic activity to two new human neuroblastoma cell lines. Cancer Res. 37: 1364-1371, 1977.

B436. Seidegard, J., Moron, M., Eriksson, L. and DePierre, J.W.: The topology of epoxide hydratase and benzpyrene monooxygenase in the endoplasmic reticulum of rat liver. Biochim. Biophys. Acta (In Press).

B437. Selkirk, J.K.: Chemical carcinogenesis: A brief overview of the mechanism of action of polycyclic hydrocarbons, aromatic amines, nitrosamines and aflatoxine. In Slaga, T.J. (Ed.): Modifiers of Chemical Carcinogenesis. (In Press).

B438. Selkirk, J.K.: Chemical Carcinogenesis. In Wolff, M. (Ed.): Burger's Medicinal Chemistry, Fourth Edition. (In Press).

B439. Selkirk, J.K.: Divergence of metabolic activation systems for short-term muragenesis assays. Nature 270: 604-607, 1977.

B440. Selkirk, J.K. and MacLeod, M.: Polycyclic hydrocarbon activation in rodent liver and fibroblasts. In Severi, L. (Ed.): Proceedings of Tumours of Early Life in Man and Animals. (In Press).

B441. Selkirk, J.K. and Wiebel, K.: Comparative metabolism of benzo(a)pyrene in rodent liver and embryonic cells in culture. Prog. Exp. Tumor Res. (In Press).

B442. Sellakumar, A., Stenback, F., Rowland, J. and Shubik, P.: Tumour induction by 7H-dibenzo(c,g)carbazole in the respiratory tract of Syrian hamsters. J. Toxicol. Environ. Health 3: 935-939, 1977.

B443. Setlow, R.B., Woodhead, A.D. and Hart, R.W.: Identical genetic material as a source for studies of cell injury. Am. J. Pathol. (In Press).

B444. Shah, M.: Thin layer chromatography (TLC) of redox reaction products of oxidative hair dyes. J. Soc. Cosmetic Chemists 28: 259-271, 1977.

B445. Shaikh, B., Huang, H. and Zielinski, W.L., Jr.: High performance liquid chromatographic analysis of supplemental vitamin E in feed. JAOAC 60: 137-139, 1977.

B446. Shaikh, B., Pontzer, N.J., Huang, S.S. and Zielinski, W.L., Jr.: Determination of N^1-methylnicotinamide in urine by high-pressure liquid chromatography. J. Chromatog. Sci. 15: 215-217, 1977.

B447. Shaikh, B. Pontzer, N.J., Molina, J.E. and Kelsey, M.I.: Separation and detection of UV-absorbing derivatives of fecal bile acid metabolites by high-performance liquid chromatography. Anal. Biochem. 85: 47-55, 1978.

B448. Shaikh, B., Huang, S.S., Pontzer, N.J. and Zielinski, W.L., Jr.: Separation of methylated purines by high pressure liquid chromatography. J. Liq. Chromatogr. 1(1): 75-88, 1978.

B449. Shain S.A. and Boesel, R.W.: Androgen receptor content of the normal and hyperplastic canine prostate. J. Clin. Invest. 61: 564-600, 1978.

B450. Shain, S.A., Boesel, R.W., Lamm, D.L. and Radwin, H.R.: Characterization of unoccupied (R) and occupied (RA) androgen binding components of the hyperplastic human prostate. Steroids (In Press).

B451. Shain, S.A., McCullough, B., Nitchuk, W.M. and Boesel, R.W.: Prostate carcinogenesis in the AXC rat. Oncology 34: 114-122, 1977.

B452. Shain, S.A., Nitchuk, W.M. and McCullough, B.: C_{19}-Steroid metabolism by spontaneous adenocarcinoma of the AXC rat ventral prostate. J. Natl. Cancer Inst. 58: 747-752, 1977.

B453. Shellabarger, C.J.: Modifying factors in rat mammary gland carcinogenesis. In Yuhas, J.M., Tennant, R.W. and Regan, J.D. (Eds.): Biology of Radiation Carcinogenesis. New York, Raven Press, 1976, pp. 31-43.

B454. Shellabarger, C.J., Stone, J.P. and Holtzman, S.: Synergism between neutron radiation and diethylstilbestrol in the production of mammary adenocarcinomas in the rat. Cancer Res. 36: 1019-1022, 1976.

B455. Shih, T-W. and Hill, D.L.: Selective inhibition and kinetics of enzymatic reactions leading to irreversible binding of benzo(a)pyrene to microsomal macromolecules of mouse lung. Cancer Biochem. Biophys. 2: 55-58, 1977.

B456. Shooter, K.V., Osborne, M.R. and Harvey, R.G.: The interaction of the 7,8-dihydrodiol-9,10-oxides of benzo(a)pyrene with bacteriophages R17 and T7. Chem. Biol. Interact. 19: 215-223, 1977.

B457. Shubik, P. and Shubik, V.: Living in a sea of carcinogens. Harper's 252: 46, 1976.

B458. Silverman, S.J. and Andrews, A.W.: Bile acids: Co-mutagenic activity using the Salmonella mammalian-microsome mutagenicity test. J. Natl. Cancer Inst. 59: 1557-1559, 1977.

B459. Singer, G.M., Taylor, H.W. and Lijinsky, W.: Liposolubility as an aspect of nitrosamine carcinogenicity; quantitative correlations and qualitative observations. Chem. Biol. Interact. 19: 133-142, 1977.

B460. Singer, S.S., Lijinsky, W. and Singer, G.M.: Transnitrosation: An important aspect of the chemistry of aliphatic nitrosamines. Tetrahedron Lett. 1613-1616, 1977.

B461. Slaga, T.J., Huberman, E., Selkirk, J.K., Harvey, R.G. and Bracken, W.M.: Carcinogenicity and mutagenicity of benzo(a)anthracene diols and diol epoxides. Cancer Res. (In Press).

B462. Synder, C.A., Erlichman, M.N., Goldstein, B.D. and Laskin, S.: An extraction method for determination of benzene in tissue by gas chromatography. Am. Ind. Hyg. Assoc. J. 38: 272-276, 1977.

B463. Synder, C.A., Goldstein, B.D., Sellakumar, A., Wolman, S.R., Bromberg, I., Erlichman, M.N. and Laskin, S.: The hematotoxicity of inhaled benzene. I. Sprague-Dawley rats and AKR mice at 300 ppm. J. Toxicol. Environ. Health. (In Press).

B464. Synder, C.A., Laskin, S. and Goldstein, B.: An extractive method for the determination of benzene in blood by gas chromatography. Am. Ind. Hyg. Assoc. J. 36(11): 833-836, 1975.

B465. Spandidos, D.A. and Siminovitch, L.: DNA-mediated gene transfer in Chinese hamster ovary cells. Cell. (In Press).

B466. Spandidos, D.A. and Siminovitch, L.: Genetic analysis by chromosome-mediated gene transfer in hamster cells. Brookhaven Symp. Biol. 29: 127-134, 1977.

B467. Spandidos, D.A. and Siminovitch, L.: Genetic analysis of malignancy using gene transfer mechanisms and mutation frequency studies. In Chandra, P. (Ed.): Antiviral Mechanisms for the Control of Neoplasia. (In Press).

B468. Spandidos, D.A. and Siminovitch, L.: Linkage of markers controlling consecutive biochemical steps in CHO cells as demonstrated by chromosome transfer. Cell 12: 235-242, 1977.

B469. Spandidos, D.A. and Siminovitch, L.: The relationship between transformation and somatic mutation in human and Chinese hamster cells. Cell 13: 651-662, 1978.

B470. Spandidos, D.A. and Siminovitch, L.: Transfer of anchorage independence by isolated metaphase chromosomes in hamster cells. Cell 12: 675-682, 1977.

B471. Spandidos, D.A. and Siminovitch, L.: Transfer of co-dominant markers by isolated metaphase chromosomes in Chinese hamster ovary cells. Proc. Natl. Acad. Sci. USA 74: 3480-3484, 1977.

B472. Spandidos, D.A. and Siminovitch, L.: Transfer of the hamster gene for hypoxanthine phosphoribosyltransferase by isolated hamster metaphase chromosomes into Lesch-Nyhan human cells. Som. Cell Genet. (In Press).

B473. Spandidos, D.A. and Siminovitch, L.: Transfer of the marker for the morphologically transformed phenotype by isolated metaphase chromosomes in hamster cells. Nature 271: 259-261, 1978.

B474. Speier, J.L., Lan, L.K.T. and Wattenberg, L.W.: Effects of administration to mice of butylated hydroxyanisole by oral intubation on benzo(a)-pyrene induced pulmonary adenoma formation and metabolism of benzo(a)pyrene. J. Natl. Cancer Inst. 60: 605-609, 1978.

B475. Sperry, J.F., Appleman, M.D. and Wilkins, T.D.: Requirement of heme for growth of Bacteroides fragilis. Appl. Environ. Microbiol. 34: 386-390, 1977.

B476. Springer, J.P., Buchi, G., Kobbe, B., Demain, A.L. and Clardy, J.: The structure of ditryptophenaline - a new metabolite of Aspergillus Flavus. Tetrahedron Lett. 28: 2403-2406, 1977.

B477. Stanley, P. and Siminovitch, L.: Complementation bewteen mutants of CHO cells resistant to a variety of plant lectins. Som. Cell Genet. 3: 391-405, 1977.

B478. Stanners, C.P.: Characterization of temperature sensitive mutants of animals cells. J. Cell Physiol. (In Press).

B479. Stanners, C.P.: Mutants and growth control. In Drewinko, B. and Humphrey, R.M. (Eds.): Regulation of Normal and Malignant Cells. Baltimore, Williams and Wilkins Company, 1977, pp. 35-47.

B480. Stanners, C.P., Wightman, T.M. and Harkins, J.L.: The effect of extreme amino acid starvation on the protein synthetic machinery of CHO cells. J. Cell. Physiol. (In Press).

B481. Steele, V.E., Marchok, A.C. and Nettesheim, P.: An organ culture-cell culture system for studying multistage carcinogenesis in respiratory epithelium. In Slaga, T.J., Sivak, A. and Boutwell, R.K. (Eds.): Mechanisms of Tumor Promotion and Cocarcinogenesis. (In Press).

B482. Steele, V.E., Marchok, A.C. and Nettesheim, P.: Transformation of tracheal epithelium exposed in vitro to N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). Int. J. Cancer 20: 234, 1977.

B483. Stenback, F.: Life history and histopathology of ultra-violet light-induced skin tumors. Natl. Cancer Inst. Monogr. (In Press).

B484. Steuer, A.F., Hentosh, P.M., Diamond, L. and Ting, R.C.: Survival differences exhibited by normal and transformed rat liver epithelial cell lines in the aggregate form. Cancer Res. 37: 1864-1867, 1977.

B485. Steuer, A.F., Rhim, J.S., Hentosh, P.M. and Ting, R.C.: Survival of human cells in the aggregate form: Potential index of in vitro cell transformation. J. Natl. Cancer Inst. 58: 917-921, 1977.

B486. Steuer, A.F. and Ting, R.C.: Current methods for monitoring in vitro carcinogenesis. In Gallo, R.C. (Ed.): Recent Advances in Cancer Research: Cell Biology, Molecular Biology, and Tumor Virology. West Palm Beach, CRC Press, Inc., 1977, pp. 67-77.

B487. Stonington, O.G., Szwec, N. and Webber, M.M.: Identification of cultured human prostatic epithelial cells. Natl. Cancer Inst. Monogr. (In Press).

B488. Suit, J., Rogers, A.E., Jetten, M.E.R. and Luria, S.E.: Effects of diet on conversion of aflatoxin B_1 to bacterial mutagens by rats in vivo and by rat's hepatic microsomes in vitro. Mutat. Res. 46: 313-323, 1977.

B489. Suzuki, Y. and Arai, Y.: Induction of estrogen-independent persistent vaginal cornification in cyproterone acetate(AC)-induced feminized male mice. Anat. Embryol. 151: 119-125, 1977.

B490. Taguchi, O., Nishizuka, Y. and Takasugi, N.: Irreversible lesions in female reproductive tracts of mice after prenatal exposure to testosterone propinate. Endocrinol. Jpn. 24: 385-391, 1977.

B491. Takahashi, M. and Pour, P.: Spontaneous alterations in the pancreas of the aging Syrian golden hamster. J. Natl. Cancer Inst. 60: 355-364, 1978.

B492. Takahashi, M. and Pour, P.: The results of pancreatography during pancreatic carcinogenesis. Am. J. Pathol. (In Press).

B493. Takahashi, M., Pour, P., Althoff, J. and Donnelly, T.: Sequential alteration of the pancreas during carcinogenesis in Syrian hamsters by N-nitrosobis(2-oxopropyl)amine. Cancer Res. 37: 4602-4607, 1977.

B494. Takahashi, M., Pour, P., Althoff, J. and Donnelly, T.: The pancreas of the Syrian hamster (Mesocricetus auratus). I. Anatomical study. Lab. Anim. Sci. 27: 336-342, 1977.

B495. Talavera, A. and Basilico, C.: Temperature sensitive mutants of BHK cells affected in cell cycle progression. J. Cell. Physiol. 92: 425-436, 1977.

B496. Tannenbaum, S.R., Archer, M.C., Wishnok, J.S. and Bishop, W.W.: Nitrosamine formation in saliva. J. Natl. Cancer Inst. 60: 251-253, 1978.

B497. Tannenbaum, S.R., Archer, M.C., Wishnok, J.S., Correa, P., Cuello, C. and Haenszel, W.: Nitrate and the etiology of gastric cancer. In Hiatt, H.H., Watson, J.D. and Winsten, J.A. (Eds.): Origins of Human Cancer. New York, Cold Spring Harbor Laboratory, 1977. pp. 1609-1625.

B498. Tannenbaum, S.R., Weisman, M. and Fett, D.: The effect of nitrate intake on nitrite formation in human saliva. Food Cosmet. Toxicol. 14: 549-552, 1976.

B499. Tannenbaum, S.R., Wishnok, J.S., Hovis, J.S. and Bishop, W.W.: N-Nitroso compounds from the reaction of primary amines with nitrite and thiocyanate. In Walker, E.A. and Griciute, L. (Eds.): Environmental N-Nitroso Compounds Analysis and Formation. IARC Scientific Publications. (In Press).

B500. Tarquini, B., Gheri, R., Romano, S., Costa, A., Cagnoni, M. and Halberg, F.: Circadian mesorhyperprolactinemia in fibrocystic mastopathy. Chronobiologia. (In Press).

B501. Theiss, J.C. and Shimkin, M.B.: Inhibiting effect of caffeine on the strain A mouse pulmonary tumor response to urethan. Cancer Res. (In Press).

B502. Thilly, W.G., Liber, H.L. and Wogan, G.N.: Toxicity and sturcture-activity relationships of cytochalasins. In Tannenbaum, S.W. (Ed.): Biochemistry and Cell Biology of Cytochalasins. (In Press).

B503. Tomaszewski, J.E., Manning, W.B. and Muschik, G.M.: A facile synthesis of benz(a)anthracene-7,12-diones. Tetrahedron Lett. 11: 971-974, 1977.

B504. Toth, B. and Nagel, D.: Tumor induction with trimethylhydrazine hydrochloride in the Syrian golden hamster. J. Natl. Cancer Inst. 59: 431-433, 1977.

B505. Toth, B., Nagel, D., Patil, K., Erickson, J. and Antonson, K.: Tumor induction with the N'-acetyl derivative of 4-hydroxy-methylphenylhydrazine, a metabolite of agaritine of Agaricus bisisporus. Cancer Res. 38: 177-180, 1978.

B506. Toth, B., Tompa, A. and Patil, K.: Tumorigenic effect of 4-methylphenylhydrazine hydrochloride in Swiss mice. Z. Krebsforsch. 89: 245-252, 1977.

B507. Ullman, B., Gudas, L.J., Cohen, A. and Martin, D.W., Jr.: Deoxyadenosine metabolism and cytotoxicity in cultured mouse T-lymphoma cells: A model for immunodeficiency disease. Cell. (In Press).

B508. Ullman, B., Lee, M., Martin, D.W., Jr. and Santi, D.V.: Cytotoxicity of 5-fluoro-2'-deoxyuridine: Requirement for reduced folate cofactors and antagonism by methotrexate. Proc. Nat. Acad. Sci. USA 75: 980-983, 1978.

B509. Upton, A.C., Beebe, G.W., Brown, J.M., Quimby, E.H. and Shellabarger, C.J.: Report of NCI Ad Hoc Working Group on the risks associated with mammography in mass screening for the detection of breast cancer. J. Natl. Cancer Inst. 59: 481-493, 1977.

B510. Vercellotti, J.R., Salyers, A.A., Bullard, W.S. and Wilkins, T.D.: Breakdown of mucin and plant polysaccharides in the human colon. Can. J. Biochem. 55: 1190-1196, 1977.

B511. Vercellotti, J.R., Salyers, A.A. and Wilkins, T.D.: Complex carbohydrate degradation in the human colon. Am. J. Clin. Nutr. (In Press).

B512. Viaje, A., Slaga, T.J., Wigler, M.H. and Weinstein, I.B.: Effects of anti-inflammatory agents on mouse skin tumor promotion, epidermal DNA synthesis, phorbol ester-induced cellular proliferation, and production of plasminogen activator. Cancer Res. 37: 1530-1536, 1977.

B513. Villereal, M.L. and Cook, J.S.: Role of the membrane potential in serum-stimulated uptake of amino acid in a diploid human fibroblast. J. Supramol. Struct. 6: 179-189, 1977.

B514. Vogel, T., Gluzman, Y. and Winocour, E.: Recombination between endogenous and exogenous SV40 genes: II. Biochemical evidence for genetic exchange. J. Virol. 24: 541-550, 1977.

B515. Walters, C.L.: Nitrosamines-environmental carcinogens? Chemistry in Britain 13: 140-145, 1977.

B516. Wang, C.C. and Hill, D.L.: Retinoid acid esterase activities: Tissue and subcellular distribution in mice. Biochem. Pharmacol. 26: 947-950, 1977.

B517. Warren, B.A., Shubik, P., Wilson, R., Garcia, H. and Feldman, R.: The microcirculation in melanomas: A correlation of in vivo microscopy and scanning electron microscopy of two melanoma tumors of hamsters. Br. J. Pathol. (In Press).

B518. Watanabe, K., Narisawa, T., Wong, C.Q. and Weisburger, J.H.: Inhibiting effect of bile acids and neutral sterols on benz(a)pyrene-induced skin tumorigenesis in mice. J. Natl. Cancer Inst. (In Press).

B519. Waters, R., Hernandez, O., Yagi, H., Jerina, D.M. and Regan, J.D.: Postreplication repair of DNA in human fibroblasts following UV irradiation or treatment with metabolites of benzo(a)pyrene. Chem. Biol. Interact. (In Press).

B520. Wattenberg, L.W.: Inhibition of chemical carcinogenesis by antioxidants. In Slaga, T.J. (Ed.): Modifiers of Chemical Carcinogenesis. (In Press).

B521. Wattenberg, L.W., Lam, L.K., Speier, J.L., Loub, W.D. and Borchert, P.: Inhibitors of chemical carcinogenesis. In Hiatt, H.H., Watson, J.D. and Winsten, J.A. (Eds.): Origins of Human Cancer. New York, Cold Spring Harbor Laboratory, 1977, pp. 785-799.

B522. Weinstein, I.B.: Current concepts on mechamisms of chemical carcinogenesis. Cancer Bull. 29: 144-152, 1977.

B523. Weinstein, I.B.: Modulation of the expression of the cancer cell phenotype. In Day, S.B. (Ed.): Cancer Invasion and Metastasis; Biologic Mechanisms and Therapy. (In Press).

B524. Weinstein, I.B.: Panel No. 6, Multiple factor etiology, chemical viral interactions in carcinogenesis. In Niebergs, H.E. (Ed.): Proceedings of the 3rd International Symposium on Detection and Prevention of Cancer, Part I, Vol. II. New York, Marcel Dekker, 1978, pp. 1609-1620.

B525. Weinstein, I.B.: Types of interaction between carcinogens and nucleic acids. In Mecanismes D'Alteration et de Reparation du DNA, Relations Avec la Mutagenese a la et la Cancerogenese Chimique. Paris, Centre National de la Recherche Scientifique, 1977, pp.2-40.

B526. Weinstein, I.B. and Troll, W.: Summary: National Cancer Institute Workshop on Tumor Promotion and Co-factors in Carcinogenesis. Cancer Res. 37: 3461-3463, 1977.

B527. Weinstein, I.B. and Wigler, M.H.: Cell culture studies provide new information on tumor promoters. Nature 270: 659-670, 1977.

B528. Weinstein, I.B., Wigler, M.H., Fisher, P.B., Sisskin, E. and Pietropaolo, C.: Cell culture studies on the biologic effects of tumor promotors. In Slaga, T.J., Sivak, A. and Boutwell, R.K. (Eds.): Mechanisms of Tumor Promotion and Cocarcinogenesis. (In Press).

B529. Weinstein, I.B., Wigler, M.H. and Pietropaolo, C.: Action of tumor promoting agents in cell culture. In Hiatt, H.H., Watson, J.D. and Winsten, J.A. (Eds.): Origins of Human Cancer. New York, Cold Spring Harbor Laboratory, 1977, pp. 751-772.

B530. Wesiburger, J.H.: Current views on mechanisms concerned with the etiology of cancers in the digestive tract. In Farber, E. et al. (Eds.): Pathophysiology of Carcinogenesis in Digestive Organs. Baltimore, University of Tokyo Press and University Park Press, 1977, pp. 1-20.

B531. Weisburger, J.H., Cohen, L.A. and Wynder, E.L.: On the etiology and metabolic epidemiology of the main human cancers. In Proceedings of the 7th Annual Conference on Environmental Toxicology. Wright-Patterson Air Force Base, Aerospace Medical Research Laboratory, 1977, pp.13-64.

B532. Weisburger, J.H., Reddy, B.S. and Wynder, E.L.: Colon cancer: Its epidemiology and experimental production. Cancer 40: 2414-2420, 1977.

B533. Weisburger, J.H., Wynder, E.L. and Cohen, L.A.: On the etiology and metabolic epidemiology of the main human cancers. In Hiatt, H.H., Watson, J.D. and Winsten, J.A. (Eds.): Origins of Human Cancer. New York, Cold Spring Harbor Laboratory, 1977, pp. 567-602.

B534. Weiss, J., Hecht, S.S., Loy, M. and Hoffmann, D.: Synthesis of the carbon-14 labelled carcinogens 5-methylchrysene, 2-methylaniline and 3-methyl-2-naphthylamine. J. Labelled Compd. Radiopharm. 14: 119-131, 1978.

B535. Wigler, M.H., DeFeo, D. and Weinstein, I.B.: Induction of plasminogen activator in cultured cells by macrocyclic plant diterpene esters and other agents related to tumor promotion. Cancer Res. (In Press).

B536. Wilding, G., Paigen, B. and Vesell, E.S.: Genetic control of inter-individual variations in racemic warfarin binding to plasma and albumin of twins. Clin. Pharmacol. Ther. 22: 831-842, 1977.

B537. Williams, C., Levine, H., Teslow, T.N. and Halberg, F.: Rhythms revealed by automatically recorded and self-measured human blood pressure during three menstrual cycles. In Proceedings of the Symposium on the Menstrual Cycle. (In Press).

B538. Williams, G.M.: The detection of chemical carcinogens by unscheduled DNA synthesis in rat liver primary cell cultures. Cancer Res. 37: 1845-1851, 1977.

B539. Winocour, E., Oren, M., Lavi, S., Vogel, T. and Gluzman, Y.: Recombination events between simain virus 40 and the host genome. In Schultz, J. and Brada, Z. (Eds.): Genetic Manipulation as it Affects the Cancer Problem. New York, Academic Press, 1977, Vol. 14, pp. 181-194.

B540. Wishnok, J.S.: Formation of nitrosamines in food and in the digestive system. J. Chem. Educ. 54: 440-442, 1977.

B541. Wishnok, J.S., Rogers, A.E., Sanchez, O. and Archer, M.C.: Dietary effects on the pharmacokinetics of three carcinogenic nitrosamines. Toxicol. Appl. Pharmacol. 43: 391-398, 1978.

B542. Wishnok, J.S. and Tannenbaum, S.R.: An unknown salivary morpholine metabolite. Anal. Chem. 49: 715A-717A, 1977.

B543. Wogan, G.N.: Food-borne environmental toxicants. In Tedeschi, C. (Ed.): Forensic Medicine: A Study in Trauma and Environmental Hazards. (In Press).

B544. Wogan, G.N.: Mycotoxins and other naturally occurring carcinogens. In Kraybill, H.F. and Mehlman, M.A. (Eds.): Nutritional and Safety Aspects of Food Processing. New York, Marcel Dekker, 1977, pp. 263-290.

B545. Woodhead, A.D.: Fish in studies of aging. Exp. Gerontol. (In Press).

B546. Woodhead, A.D.: Aging changes in the liver of two Poeciliid fishes, the guppy Poecilia (Lebistes) Reticulata and the Amazon Molly, P. Formosa. Exp. Gerontol. 13: 37-45, 1978.

B547. Woodhead, A.D. and Scully, P.M.: A comparative study of the pre-tumorous thyroid gland of the gynogenetic teloest, Poecilia formosa, and that of other Poeciliid fishes. Cancer Res. 37: 3751-3755, 1977.

B548. Woodhead, A.D., Setlow, R.B. and Hart, R.W.: Genetically uniform strains of fish as laboratory models for experimental studies of the effects of ionizing radiation. IAEA Technical Report. (In Press).

B549. Woodhead, A.D., Setlow, R.B. and Hart, R.W.: The development of thyroid neoplasia in old age in the Amazon Molly, Poecilia Formosa. Exp. Gerontol. 12: 193-200, 1977.

B550. Woodhead, A.D., Setlow, R.B. and Hart, R.W.: Tissue changes resulting from the injection of γ-irradiated cells into the gynogenetic teleost, Poecilia Formosa. Cancer Res. 37: 4261-4266, 1977.

B551. Worton, R.G., Ho, C.C. and Duff, C.: Chromosome stability in CHO cells. Som. Cell Genet. 3: 27-45, 1977.

B552. Wright, L.H., Chan, J.A., Schroer, J.A. and Aszalos, A.A.: A ^{13}C nuclear magnetic resonance study of N-acetyl daunorubicinol. J. Org. Chem. 42: 2344-2345, 1977.

B553. Wynder, E.L. and Reddy, B.S.: Colon cancer prevention: Today's challenge to biomedical scientists and clinical investigators. Cancer 40: 2565-2571, 1977.

B554. Wynder, E.L. and Reddy, B.S.: Diet and cancer of the colon. In Winick, M. (Ed.): Nutrition and Cancer. New York, John Wiley & Sons, Inc., 1977, pp.57-71.

B555. Wynder, E.L. and Reddy, B.S.: Epidemiology of cancer of the colon: Incidence, diet and metabolic factors. In Enker, W.E. (Ed.): Carcinoma of the Colon and Rectum. (In Press).

B556. Yamasaki, H., Fibach, E., Nudel, U., Weinstein, I.B., Rifkind, R.A. and Marks, P.A.: Tumor promoters inhibit spontaneous and induced differentiation of murine erythroleukemia cells in culture. Proc. Natl. Acad. Sci. 74(8): 3451-3455, 1977.

B557. Yamasaki, H., Fibach, E., Weinstein, I.B., Nudel, U., Rifkind, R.A. and Marks, P.A.: Inhibition of Friend leukemia cell differentiation by tumor promoters. In Proceedings of the Oji International Seminar. (In Press).

B558. Yanagi, K., Talavera, A., Nishimoto, T. and Rush, M.G.: Inhibition of Herpes simplex virus type I replication in temperature-sensitive cell cycle mutants. J. Virol. 25: 42-50, 1978.

B559. Yamasaki, H., Huberman, E. and Sachs, L.: Metabolism of the carcinogenic hydrocarbon benzo(a)pyrene in human fibroblast and epithelial cells. Int. J. Cancer 19: 378-382, 1977.

B560. Yang, H.S., Okun, J.D. and Archer, M.C.: Nonenzymatic microbial acceleration of nitrosamine formation. J. Agric. Food Chem. 25: 1181-1183, 1977.

B561. Yeh, C.H., Fu, P.P., Beland, F.A. and Harvey, R.G.: Application of CNDO/2 theoretical calculations to interpretation of the chemical reactivity and biological activity of the Syn and Anti diolepoxides of benzo(a)pyrene. Bioorg. Chem. (In Press).

B562. Yoshida, A., Iqbal, Z.M. and Epstein, S.S.: Hepatocarcinogenic effects of N-nitroso-N-methylurea in guinea pigs. Cancer Res. 37: 4034-4048, 1977.

B563. Zacharias, D.E., Glusker, J.P., Harvey, R.G. and Fu, P.P.: Molecular structure of the K-region Cis-dihydrodiol of 7,12-dimethylbenz(a)anthracene. Cancer Res. 37: 775-781, 1977.

B564. Zwilling B.S., Springer, S.T. and Kaufman, D.G.: Effect of systemic administration of BCG cell walls on bronchogenic carcinoma in hamsters. J. Natl. Cancer Inst. 58: 1473-1477, 1977.

C. INTRAMURAL/COLLABORATIVE PROGRAMS[1]

C1. Autrup, H., Barrett, L.A., Jackson, F.E., Jesudason, M.L., Stoner, G.D., Phelps, P., Trump, B.F. and Harris, C.C.: Explant culture of human colon. Gastroenterology. (In Press).

C2. Autrup, H., Harris, C.C., Fugaro, S. and Selkirk, J.K.: Effect of bile acids and other chemicals on the metabolism of benzo(a)pyrene by cultured rat colon. Chem. Biol. Interact. 18: 337-347, 1977.

C3. Autrup, H., Harris, C.C., Stoner, G.D., Jesudason, M.L. and Trump, B.F.: Binding of chemical carcinogens to macromolecules in cultured human colon. J. Natl. Cancer Inst. 59: 351-355, 1977.

C4. Autrup, H., Harris, C.C., Stoner, G.D., Selkirk, J.K., Schafer, P. and Trump, B.F.: Metabolism of ^{3}H-benzo(a)pyrene by cultured human pulmonary alveolar macrophages. Lab. Invest. 38: 217-223, 1978.

C5. Autrup, H., Stoner, G.D., Jackson, F.E., Harris, C.C., Shamsuddin, T., Trump, B.F. and Barrett, L.A.: Explant culture of rat colon: A model system for studying metabolism of chemical carcinogens. In Vitro. (In Press).

C6. Barrett, L.A., McDowell, E., Harris, C.C. and Trump, B.F.: Studies on ischemic cell injury: X. Reversal of ischemic cell injury in hamster trachea and human bronchus by organ culture. Beitr. Pathol. 161: 109-122, 1977.

C7. Barrett, L.A., McDowell, E.M., Hill, T.A., Pyeatte, J., Harris, C.C. and Trump, B.F.: Induction of atypical squamous metaplasia with benzo(a)pyrene in cultured hamster tracheas. Eur. J. Cancer. (In Press).

C8. Berman, J., Rice, J.M. and Strandberg, J.: Granular cell variants in a rat schwannoma, evidence of neurogenic origin of granular cell tumor (myoblastoma). Vet. Pathol. (In Press).

C9. Berman, J., Stoner, G.D., Dawe, C., Rice, J.M. and Kingsbury, E.: Histochemical demonstration of collagen fibers in ascorbic acid-fed cell cultures. In Vitro. (In Press).

C10. Casto, B.C. and DiPaolo, J.A.: Biological activity of polycyclic hydrocarbons for Syrian hamster cells in vitro. In Ts'o, P.O.P. and Gelboin, H.V. (Eds.): Polycyclic Hydrocarbons and Cancer: Chemistry, Molecular Biology and Environment. (In Press).

C11. Casto, B.C., Janoski, N. and DiPaolo, J.A.: Development of a focus assay model for transformation of hamster cells in vitro by chemical carcinogens. Cancer Res. 37: 3508-3515, 1977.

---

[1]Papers published jointly with Intramural and Collaborative Program's staff.

C12. DiPaolo, J.A. and Casto, B.C.: Chemical carcinogenesis. In Gallo, R.C. (Ed.): Recent Advances in Cancer Research: Cell Biology, Molecular Biology, and Tumor Virology. Cleveland, CRC Press, Inc., Vol. I, 1977, pp. 17-47.

C13. DiPaolo, J.A. and Casto, B.C.: In vitro carcinogenesis with cell strains in early passages. In Sanford, K. (Ed.): Third Decennial Review Conference on Cell Tissue and Organ Culture. Gene Expression and Regulation in Cultured Cells. Natl. Cancer Inst. Monogr. (In Press).

C14. DiPaolo, J.A. and Casto, B.C.: In vitro transformation of mammalian cells: Sequential treatment with diverse agents. In Slaga, T.J., Sivak, A. and Boutwell, R.K. (Eds.): Mechanisms of Tumor Promotion and Cocarcinogenesis. (In Press).

C15. Gaffield, W., Roller, P.P., Palmer, W.G. and Keefer, L.K.: Tritium gas exposure as an alternative to base-catalyzed exchange for the one-step tritiation of nitrosamines. J. Labeled Compd. Radiopharm. 14: 91-97, 1978.

C16. Gelboin, H.V., Selkirk, J.K., Okuda, T., Nemoto, N., Yang, S.K., Wiebel, F., Whitlock, J.P., Jr., Rapp, H.J. and Bas, R.C., Jr.: Benzo(a)pyrene metabolism: enzymatic and liquid chromatographic analysis and application to human liver lymphocytes and monocytes. In Jallow, D.J. (Ed.): Biological Reactive Intermediates. New York, Plenum Press, 1977, pp. 98-124.

C17. Grubbs, C.J., Moon, R.C., Norikane, K., Thompson, H.J. and Becci, P.J.: 1-Methyl-1-nitrosourea-induction of cancer in a localized area of the Syrian golden hamster trachea. In Homburger, F. (Ed.): Symposium on the Syrian Hamster in Toxicology and Carcinogenesis Research. (In Press).

C18. Grubbs, C.J., Moon, R.C., Squire, R.A., Farrow, G.M., Stinson, S.F., Goodman, D.G., Brown, C.C. and Sporn, M.B.: 13-cis-retinoic acid: Inhibition of bladder carcinogenesis induced in rats by N-butyl-N-(4-hydroxybutyl)nitrosamine. Science 198: 743-744, 1977.

C19. Harris, C.C., Autrup, H., Stoner, G.D., Barrett, L.A., McDowell, E.M., Trump, B.F. and Schafer, P.: Binding of dimethylnitrosamine and 1,2-dimethylhydrazine to macromolecules in cultured human bronchi. Cancer Res. 37: 2309-2311, 1977.

C20. Harris, C.C., Autrup, H., Stoner, G.D., McDowell, E.M., Trump, B.F. and Schafer, P.: Metabolism of acyclic and cyclic N-nitrosamines in cultured human bronchi. J. Natl. Cancer Inst. 59: 1401-1406, 1977.

C21. Harris, C.C., Autrup, H., Stoner, G.D. and Trump, B.F.: Carcinogenesis studies in human respiratory epithelium: An experimental model system. In Harris, C.C. (Ed.): Pathogenesis and Therapy of Lung Cancer. (In Press).

C22. Harris, C.C., Autrup, H., Stoner, G.D. and Trump, B.F.: Model systems using human lung for carcinogenesis studies. In Harris, C.C. (Ed.): Pathogenesis and Therapy of Lung Cancer. (In Press).

C23. Harris, C.C., Autrup, H., Stoner, G.D., Valerio, M., Fineman, E., Schafer, P., Barrett, L.A., McDowell, E.M. and Trump, B.F.: Explant culture and xenotransplantation of human bronchi. In Saffiotti, U. and Autrup, H. (Eds.): In Vitro Carcinogenesis Guide to the Literature, Recent Advances and Laboratory Procedures. Carcinogenesis Program, DCCP, NCI: Technical Report Series No. 44. U.S. DHEW, PHS, NIH, NCI. DHEW Publ. No. (NIH) 78-844. Wash.,D.C., U.S. Govt. Print. Off., 1978, pp. 144-153.

C24. Harris, C.C., Autrup, H., Stoner, G.D., Yang, S.K., Leutz, J., Gelboin, H.V., Selkirk, J.K., Connor, R.C., Barrett, L.A., Jones, R.T., McDowell, E.M. and Trump, B.F.: Metabolism of benzo(a)pyrene and 7,12-dimethylbenz(a)anthracene in cultured human bronchus and pancreatic duct. Cancer Res. 37: 3349-3355, 1977.

C25. Harris, C.C., Autrup, H., van Haaften, C., Connor, R.C., Frank, A., Barrett, L.A., McDowell, E.M. and Trump, B.F.: Inhibition of benzo(a)pyrene binding to DNA in cultured human bronchi. In Nieburgs, H. (Ed.): Proceedings of the 3rd International Symposium on Cancer Detection and Prevention. Amsterdam, Excerpta Medica, 1978, pp. 1359-1366.

C26. Harris, C.C.,Hsu, I.C., Stoner, G.D., Trump, B.F. and Selkirk, J.K.: Human pulmonary alveolar macrophages metabolize benzo(a)pyrene to proximate and ultimate mutagens. Nature. (In Press).

C27. Harris, C.C., Saffiotti, U. and Trump, B.F.: Meeting report: Carcinogenesis studies in human cells and tissues. Cancer Res. 38: 474-475, 1978.

C28. Hatch, G.G., Balwierz, P.J., Casto, B.C. and DiPaolo, J.A.: Characteristics of hamster cells transformed by the combined action of chemical and virus. Int. J. Cancer 21: 121-127, 1978.

C29. Hsu, I.C., Autrup, H., Stoner, G.D., Trump, B.F., Selkirk, J.K. and Harris, C.C.: Human bronchus-mediated mutagenesis of mammalian cells by carcinogenic polynuclear aromatic hydrocarbons. Proc. Natl. Acad. Sci. (In Press).

C30. Huberman, E., Yang, S.K., McCourt, D.W. and Gelboin, H.V.: Mutagenicity of (+) and (-) trans-7,8-dihydroxy-7,8-dihydrobenzo(a)pyrenes and the hydrolysis and reduction products of two stereoisomeric benzo(a)pyrene 7,8-diol-9,10-epoxides to mammalian cells in culture. Int. J. Cancer (In Press).

C31. Ikenaga, M. and Kakunaga, T.: Excision of 4-nitroquinoline-1-oxide damage and transformation in mouse cells. Cancer Res. 37: 3672-3678, 1977.

C32. Jeffrey, A.M., Weinstein, I.B., Jennette, K.W., Grzeskowiak, K., Nakanishi, K., Harvey, R.G., Autrup, H. and Harris, C.C.: Structures of benzo-(a)pyrene-nucleic acid adducts formed in human and bovine bronchial explants. Nature 269: 348-350, 1977.

C33. Kakefuda, T., Lovinger, G.G., Gilden, R.V. and Hatanaka, M.: Electron microscopic studies of circular DNA in mouse embryo fibroblasts infected by Rauscher leukemia virus. J. Virol. 21: 792-795, 1977.

C34. Levitt, M.H., Harris, C.C., Squire, R.A., Springer, S.T., Wenk, M.L., Mollelo, C., Thomas, D., Kingsbury, E. and Newkirk, C.: Experimental pancreatic carcinogenesis. I. Morphogenesis of pancreatic adenocarcinoma in the Syrian golden hamster induced by N-nitroso-bis(2-hydroxypropyl)-amine. Am. J. Pathol. 88: 5-15, 1977.

C35. Levitt, M.H., Harris, C.C., Squire, R.A., Wenk, M.L., Mollelo, C., Springer, S.T. and Thomas, D.: Experimental pancreatic carcinogenesis. II. Lifetime carcinogenesis studies in the Syrian golden hamster induced by N-nitroso-bis(2-hydroxypropyl)amine. J. Natl. Cancer Inst. 60: 701-705, 1978.

C36. Lichti, U., Slaga, T.J., Ben, T.L., Patterson, E., Hennings, H. and Yuspa, S.H.: Dissociation of tumor promoter-stimulated ornithine decarbosylase activity and DNA synthesis in mouse epidermis in vivo and in vitro by fluocinolone acetonide, a tumor-promotion inhibitor. Proc. Natl. Acad. Sci. 74: 3908-3912, 1977.

C37. Love, L.A., Lijinsky, W., Keefer, L.K. and Garcia, H.: Chronic oral administration of 1-nitrosopiperazine at high doses to MCR rats. Z. Krebsforsch. 89: 69-73, 1977.

C38. Mager, R., Huberman, E., Yang, S.K., Gelboin, H.V. and Sachs, L.: Transformation of normal hamster cells by benzo(a)pyrene diol-epoxide. Int. J. Cancer 19: 814-817, 1977.

C39. McDowell, E.M., Barrett, L.A., Glavin, F., Harris, C.C. and Trump, B.F.: The respiratory epithelium. I. Human bronchus. J. Natl. Cancer Inst. (In Press).

C40. McDowell, E.M., McLaughlin, J.S., Merenyi, D.K., Kieffer, R.F., Harris, C.C. and Trump, B.F.: The respiratory epithelium. V. Histogenesis of lung carcinomas in the human. J. Natl. Cancer Inst. (In Press).

C41. Meadows, A.T., D'Angio, G.J., Mike, V., Banfi, A., Harris, C.C., Jenkin, R.D.T. and Schwartz, J.A.: Patterns of second malignant neoplasms in children. Cancer 40: 1903-1912, 1977.

C42. Milo, G.E., Blakeslee, J., Yohn, D. and DiPaolo, J.A.: Biochemical activation of AHH activity, cellular distribution of polynuclear hydrocarbon metabolites, and DNA damage by PNH products in human cells in vitro. Cancer Res. 38: 1638-1644, 1978.

C43. Moon, R.C., Grubbs, C.J., Sporn, M.B. and Goodman, D.G.: Retinyl acetate inhibits mammary carcinogenesis induced by N-methyl-N-nitrosourea. Nature 67: 620-621, 1977.

C44. Paradise, L.J., Boren, H.G., Hartley, E.W. and Harris, C.C.: Localization and retention of (^{3}H)benzo(a)pyrene in hamster respiratory epithelial cells: Quantitative autoradiography. Cancer Res. (In Press).

C45. Pawson, B.A., Cheung, H.-C., Han, R.-J.L., Trown, P.W., Buck, M., Hansen, R., Bollag, W., Ineichen, U., Pleil, H., Ruegg, R., Dunlop, N.M., Newton, D.L. and Sporn, M.B.: Dihydroretinoic acids and their derivatives. Synthesis and biological activity. J. Med. Chem. 20: 918-925, 1977.

C46. Poirier, M.C., Yuspa, S.H., Weinstein, I.B. and Blobstein, S.H.: Detection of carcinogen-DNA adducts by radioimmunoassay. Nature 270: 186-188, 1977.

C47. Rice, J.M., Joshi, S.R., Shenfelt, R.E. and Wenk, M.L.: Transplacental carcinogenic activity of 7,12-dimethylbenz(a)anthracene. In Jones, P.W. and Freudenthal, R.I. (Eds.): Carcinogenesis, Vol. 3. Polynuclear Aromatic Hydrocarbons. New York, Raven Press, 1978, pp. 413-422.

C48. Rice, J.M., London, W.T., Palmer, A.E., Sly, D.L. and Williams, G.M.: Transplacental effects of ethylnitrosourea in the patas monkey. In Proceedings of Perugia Cancer Conference on Tumors of Early Life in Man and Animals. (In Press).

C49. San, R.H.C., Rice, J.M. and Williams, G.M.: Lack of correlation between plasminogen activating factor production and tumorigenicity in rat liver epithelial cells. Cancer Lett. 3: 243-246, 1977.

C50. Schwartz, J.A., Viaje, A., Slaga, T.J., Yuspa, S.H., Hennings, H. and Lichit, U.: Fluocinolone acetonide: a potent inhibitor of mouse skin tumor promotion and epidermal DNA synthesis. Chem. Biol. Interact. 17: 331-347, 1977.

C51. Shimkin, M.B., Stoner, G.D. and Theiss, J.C.: Lung tumor response in mice to metals and metal salts. Adv. Exp. Med. Biol. 91: 85-91, 1978.

C52. Slaga, T.J., Lichti, U., Hennings, H., Elgjo, K. and Yuspa, S.H.: Effects of tumor promoters and steroidal anti-inflammatory agents on newborn mouse skin in vivo and in vitro. J. Natl. Cancer Inst. 60: 425-431, 1978.

C53. Sly, D.L., London, W.T., Palmer, A.E. and Rice, J.M.: Disseminated cryptococcosis in a patas monkey (Erythrocebus patas). Lab. Anim. Sci. 27: 694-699, 1977.

C54. Sly, D.L., London, W.T., Palmer, A.E. and Rice, J.M.: Growth and hematologic development of the patas monkey to one year of age. J. Med. Primatol. (In Press).

C55. Squire, R.A., Sporn, M.B., Brown, C.C., Smith, J.M., Wenk, M.L. and Springer, S.T.: Histopathological evaluation of the inhibition of rat bladder carcinogenesis by 13-cis-retinoic acid. Cancer Res. 37: 2930-2936, 1977.

C56. Stoner, G.D., Harris, C.C., Autrup, H., Trump, B.F. and Kingsbury, E.W.: Explant culture of human peripheral lung tissue. I. Metabolism of benzo(a)-pyrene. Lab. Invest. (In Press).

C57. Stoner, G.D., Harris, C.C., Bostwick, D.G., Jones, R.T., Trump, B.F., Kingsbury, E.W., Fineman, E. and Newkirk, C.: Isolation and characterization of epithelial cells from bovine pancreatic duct. In Vitro. (In Press).

C58. Theiss, J.C., Stoner, G.D. and Kniazeff, A.J.: Effect of reovirus infection on the strain A mouse pulmonary tumor response to urethan. J. Natl. Cancer Inst. (In Press).

C59. Theiss, J.C., Stoner, G.D., Shimkin, M.B. and Weisburger, E.K.: Test for carcinogenicity of organic contaminants of U.S. drinking waters by pulmonary tumor response in strain A mice. Cancer Res. 37: 2717-2720, 1977.

C60. Trump, B.F., McDowell, E.M., Glavin, F., Barrett, L.A., Becci, P.J., Schurch, W., Kaiser, H.E. and Harris, C.C.: The respiratory epithelium. III. Histogenesis of epidermoid metaplasia and carcinoma in situ in the human. J. Natl. Cancer Inst. (In Press).

C61. Vesselinovitch, S.D., Koka, M., Rao, K.V.N., Mihailovich, N. and Rice, J.M.: Prenatal multicarcinogenesis by ethylnitrosourea in mice. Cancer Res. 37: 1822-1828, 1977.

C62. Ward, J.M., Rice, J.M., Roller, P.P. and Wenk, M.L.: Natural history of intestinal neoplasms induced in rats by a single injection of methyl-(acetoxymethyl)nitrosamine. Cancer Res. 37: 3046-3052, 1977.

C63. Ward, J.M., Sporn, M.B., Wenk, M.L., Smith, J.M., Feeser, D. and Dean, R.J.: Dose response and histopathologic evaluation of the effect of retinoids on colon lesions induced in rats by intrarectal administration of N-methyl-N-nitrosourea. J. Natl. Cancer Inst. (In Press).

C64. Weisburger, E.K.: Bioassay program for carcinogenic hazards of cancer chemotherapeutic agents. Cancer 40: 1935-1949, 1977.

C65. Weisburger, E.K.: Carcinogenicity studies on halogenated hydrocarbons. Environ. Health Perspect. (In Press).

C66. Weisburger, E.K.: Foreword. In A Rational Evaluation of Pesticidal versus Mutagenic/Carcinogenic Action. (In Press).

C67. Weisburger, E.K., Evarts, R.P. and Wenk, M.L.: Inhibitory effect of butylated hydroxytoluene (BHT) on intestinal carcinogenesis in rats by azoxymethane. Food Cosmet. Toxicol. 15: 139-141, 1977.

C68. Weisburger, E.K., Russfield, A.B., Homburger, F., Weisburger, J.H., Boger, E., Van Dongen, C.G. and Chu, K.: Testing of 21 environmental aromatic amines or derivatives for long-term toxicity or carcinogenicity. J. Toxicol. Environ. Health (In Press).

C69. Wiebel, F.J., Brown, S., Waters, H.L. and Selkirk, J.K.: Activation of xenobiotics by monooxygenases: Cultures of mammalian cells as an analytical tool. Arch. Toxicol. (In Press).

C70. Yang, S.K., Fu, P.P., Roller, P.P., Harvey, R.G. and Gelboin, H.V.: Evidence for an unstable 3,4-epoxide as a metabolic intermediate of benz(a)-anthracene. Fed. Proc. 37: 597, 1978.

C71. Yang, S.K., Róller, P.P., Fu, P.P., Harvey, R.G. and Gelboin, H.V.: Evidence for a 2,3-epoxide as a intermediate in the microsomal metabolism of benzo(a)pyrene to 3-hydroxybenzo(a)pyrene. Biochem. Biophys. Res. Commun. 77: 1176-1182, 1977.

C72. Yuspa, S.H., Lichti, U., Hennings, H., Ben, T.L., Patterson, E. and Slaga, T.J.: Tumor promoter stimulated proliferation in mouse epidermis in vivo and in vitro: Mediation by polyamines and inhibition by the anti-promoter steroid fluocinolone acetonide. In Slaga, T.J., Sivak, A. and Boutwell, R.K. (Eds.): Mechanisms of Tumor Promotion and Cocarcinogenesis. (In Press).

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Shabad, L.M. - B372
Shah, M.J. - B360, B444
Shaikh, B. - B195, B445, B446, B447, B448
Shain, S.A. - B45, B449, B450, B451, B452
Shamsuddin, T. - C5
Sheinfil, A. - B383
Shellabarger, C.J. - B453, B454, B509
Shenefelt, R.E. - C48
Sherlock, P. - B285
Shih, T.-W. - B455
Shimada, T. - A16
Shimkin, M.B. - B501, C52, C60
Shohami, E. - B39, B234, B235
Shons, A.R. - B165
Shooter, K. V. - B456
Shubik, P. - B54, B55, B58, B297, B442, B457, B517
Shubik, V. - B457
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Simpson, H. W. - B165
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Singer, G. M. - B300, B459, B460
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Sisskin, E. - B528
Slaga, T.J. - B38, B461, B512, C37, C51, C53, C73
Slein, M.W. - B428, B429
Sly, D.L. - C49, C54, C55
Smith, D.P. - B392
Smith, J.M. - A71, A99, C56, C64
Snyder, C.A. - B462, B463, B464
Solt, D. - B123, B125, B126
Somers, D.G. - B90
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Sothern, R.B. - B168
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Sperry, J.F. - B412, B418, B475
Spindler, S.M. - B128
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Springer, S.T. - B564, C35, C36, C56
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Taguchi, O. - B345, B490
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Tannenbaum, S.R. - B151, B171, B496, B497, B498, B499, B542
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Watanabe, K. - B386, B387, B518
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ANNUAL REPORT

CARCINOGENESIS TESTING PROGRAM
DIVISION OF CANCER CAUSE AND PREVENTION

October 1, 1977 through September 30, 1978

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VI. APPENDIX
Chemicals Being Tested for Carcinogenicity

I. SUMMARY

The Carcinogenesis Testing Program (1) plans, directs, and conducts a program for the in vivo and in vitro testing of chemical and physical agents in the environment for carcinogenic and cocarcinogenic effects; (2) establishes program priorities, allocates resources, evaluates effectiveness and represents program area in management and scientific decision-making meetings in the Institute, and coordinates with other related programs in the Institute; (3) through intramural and contract resources, administers research in the development and evaluation of standardized methods, designs and models for in vivo and in vitro carcinogenesis testing, related toxicology, and tumor pathology; (4) establishes, maintains and provides technical, scientific, and management information/reporting resources for monitoring, analyzing and disseminating program findings; (5) interacts and coordinates with the Clearinghouse for Environmental Carcinogens through the Director of the Division; and (6) advises the Director of the Division on carcinogenesis testing and supports the activities of the National Cancer Advisory Board and other scientific advisory committees with regard to the program.

The Carcinogenesis Testing Program was created in July 1977 to give greater emphasis to the testing of environmental chemicals for carcinogenicity. During the first year of its existence, the highest priority was given to the analysis and reporting of a series of 207 carcinogen bioassays in animals that had been conducted previously by other National Cancer Institute (NCI) staff members. By May 1978, Program staff had completed the analyses of experiments with 134 chemicals and the reports made public. The analyses of the experiments on the remaining 73 chemicals are in progress and are scheduled for completion by September 30, 1978. Thus far, 82 chemicals have been revealed to have the potential to produce cancer.

The next highest priority was given to starting new carcinogen bioassays. During the year 134 chemicals were selected for testing and experiments were started on 60 chemicals. During the next year it is anticipated that 100 to 120 chemicals will start on test.

Another high priority activity was the ongoing validation of short-term, in vitro tests. A battery of short-term tests for bacterial and mammalian cell transformation are being explored to determine their usefulness as predictors of the carcinogen potential of chemicals. As part of these experiments, several hundred chemicals are being tested each year.

The Carcinogenesis Testing Program is also serving as a national resource for information on carcinogenesis testing. Information on chemical carcinogenesis testing is also distributed through three types of publications, technical reports, an annual survey of animal carcinogenesis tests, and Carcinogenesis Abstracts. Program staff members assist other Government agencies and other governments in the design and interpretation of carcinogen bioassays. The Program also has collaborative studies with the Environmental Protection Agency (EPA), National Institute of Environmental Health Sciences (NIEHS), and National Institute of Occupational Safety and Health (NIOSH).

II. OVERVIEW

A. INTRODUCTION

The Carcinogenesis Testing Program is the organization within the Government that is responsible for the testing of chemicals for carcinogenicity. To prevent cancer, it is necessary that those chemicals that are capable of causing cancer be identified so that measures can be taken to prevent or minimize exposure.

People are exposed to chemicals in the workplace, in the home, and throughout the environment. Of the several million chemicals in existence, about 130,000 different chemicals are in sufficient use in the United States to cause concern and more than 30,000 of these are produced in commercially significant quantities. Unfortunately, few of the chemicals to which people may be exposed have been adequately tested to learn the potential risks, if any, of cancer. Of the few thousand chemicals that have been tested (because there was suspicion that they might be carcinogenic), evidence has been obtained that is sufficient to establish the carcinogenicity of a little over 200 chemicals and suggestive evidence that requires confirmation has been obtained for another 200 chemicals. Without testing there is no way of knowing how many more environmental chemicals might be carcinogenic. Most scientists estimate, however, that far fewer than 10 percent of chemicals will be found to be carcinogens. It is important, too, that those chemicals that are not capable of producing cancer be identified so that restrictive and expensive barriers to their use need not be employed.

In the Carcinogenesis Testing Program chemicals are tested for carcinogenicity by either lifetime studies in experimental animals or by short-term experiments with cultured cells and microorganisms, or both. The only other presently available approach to detecting chemical carcinogens is through epidemiologic studies as described in the annual report of the Field Studies and Statistics Program, Division of Cancer Cause and Prevention (DCCP). It is preferable not to learn about carcinogenicity from human studies because they cannot be performed until people have been exposed to the chemicals. Our animal studies are designed to identify potentially hazardous chemicals so that human exposure can be avoided.

Historically the Carcinogenesis Testing Program evolved from NCI research programs on chemical carcinogenesis. In March 1961 a Carcinogenesis Studies Branch was established in the Field Studies Area to investigate carcinogenic factors, particularly as hazards in the human environment. Since there was an apparent need to test chemicals for carcinogenicity that was not being fulfilled by other Government agencies, a contract-supported carcinogen bioassay activity was initiated in 1962. Much of the early efforts dealt with the development of methods for bioassays. Work in Carcinogen testing gradually expanded and received even greater emphasis after 1966 when the Carcinogenesis Studies Branch developed into the Carcinogenesis Program as part of the Etiology Area, which itself was in 1972 to become the Division of Cancer Cause and Prevention of the NCI. Following

the successful development of carcinogen bioassay methods and standards in the 1960s, the contract-supported efforts were greatly expanded in the 1970s to apply carcinogen bioassay methods to the testing of hundreds of chemicals. By June 1976, more than 330 different chemicals had been tested for carcinogenicity in experimental animals.

In recognition of the importance of carcinogen testing, a separate program, the Carcinogenesis Testing Program, was created operationally in July 1976 and formally in July 1977 to give greater emphasis to the testing of chemicals for carcinogenicity. Program activities include the testing of chemicals in lifetime experiments in animals (approximately 100 chemicals started on test this year), the testing of chemicals in a battery of short-term tests, and continuation of research on methods of testing to obtain tests that are more sensitive, quicker, and less expensive. At present the Carcinogenesis Testing Program conducts about 90 percent of the Government's carcinogen testing. The Program also serves as a valuable informational and educational center for carcinogenesis testing.

B. PROGRAM PLANS

The long-term goal of the Carcinogenesis Testing Program is the identification of all the chemical carcinogens in man's environment. This task is complicated by the introduction of 600 or 700 new chemicals into commerce each year and by changing patterns of production and use of chemicals. The primary immediate objectives of the Program are to test as many chemicals in long-term tests as permitted by the resources available, to learn the predictability for carcinogenesis of short-term tests so that they can replace or augment long-term tests, and to develop improved methods for both long-term and short-term tests so that they are more sensitive, have lower error rates, and are more cost-effective.

An important element in carcinogen testing is the selection of chemicals for testing. Both formal and informal mechanisms are used to solicit nominations from other Government agencies and from the public. Following internal Program review of nominations, a subgroup of the Clearinghouse on Environmental Carcinogens advises Program on the relative priority for testing each chemical. The deliberations are conducted in public by a group of non-Government individuals who represent the various public and private sectors to insure that the chemicals selected are of great national importance. A similar dual Government and Clearinghouse review and assessment is used to design suitable animal experiments and to analyze the results of experiments. At present, Program is conducting animal bioassays at the rate of about 100 chemicals per year. Since each assay requires four or five years to complete at a cost of about $300,000, it is necessary that the chemicals be properly selected and the experiments well designed and properly carried out for maximum utilization of resources and for obtaining maximum information about the capacity of chemicals to produce cancer.

As experience has been gained in designing and conducting carcinogen bioassays, it has become apparent that the animal experiments must be expanded to

demonstrate the uptake of the chemical and to measure the doses received. When additional resources become available, more studies will be conducted on the distribution of the chemical within the body; its metabolism, storage, and excretion; and the modifying influence of host factors such as sex, age, immune status, endocrine function, diet, and genetic background. One new Program activity, initiated last year in collaboration with the National Institute of Environmental Health Sciences is the study of toxic effects (other than carcinogenicity) that may occur during life-long carcinogenicity tests in animals.

The battery of short-term in vitro tests are being validated and those tests that are sufficiently developed are being applied as mutagenicity or carcinogenicity tests. The Carcinogenesis Testing Program is not attempting to develop new short-term tests but rather to evaluate, modify if necessary, and apply those promising tests developed by others. The short-term tests include those for bacterial mutagenicity, mammalian mutagenicity, DNA damage and repair, and mammalian cell transformation. Important components are the study of human cells, epithelial cells, and enzyme activation methods. It is noteworthy that evaluating the predictability of short-term tests depends on the knowledge of the carcinogenicity or noncarcinogenicity of chemicals acquired from the long-term animal studies.

Information obtained from carcinogenicity testing has considerable impact on people's lives and on the economy, especially when the results of the Program's experiments lead to regulatory action as, for example, with the banning of the flame-retardant tris from children's sleepwear. Dissemination of the results of Program's experiments has led to a new sphere of activity, including information exchanges with regulatory agencies, industries, trade associations, unions, consumer advocate groups and foreign nations. Program maintains a Registry of Tumor Pathology and a repository of bioassay records and materials which serve as educational resources. The repository also functions as a source of legal documents and is widely used by Government and non-Government adversaries. As described below, Program also serves as an information center on carcinogenesis testing, publishes Carcinogenesis Abstracts and the Survey of Compounds Which Have Been Tested for Carcinogenic Activity (PHS 149), and collaborates with the International Agency for Research on Cancer on the international Evaluation of the Carcinogenic Risk of Chemicals to Man.

C. ORGANIZATION

OFFICE OF THE ASSOCIATE DIRECTOR FOR CARCINOGENESIS TESTING PROGRAM

R. Griesemer, D.V.M., Ph.D. - Associate Director
J. Douglas, Ph.D. - Expert

In Vitro Carcinogenesis

V. Dunkel, Ph.D.

Registry of Experimental Cancers

H. Stewart, M.D.[1]
M. Deringer, Ph.D.
B. Sass, V.M.D.
C. Hoch-Ligeti, M.D.[2]

Technical Information Branch

S. Siegel, Ph.D. - Acting Chief
J. Chase, M.S.
T. Fischetti, B.S.
M. Linhart, M.A.[3]

Toxicology Branch

C. Cueto, Jr., Ph.D. - Acting Chief
K. Chu, Ph.D.
H. Milman, Ph.D.
T. Orme, Ph.D.
C. Whitmire, Ph.D.

Tumor Pathology Branch

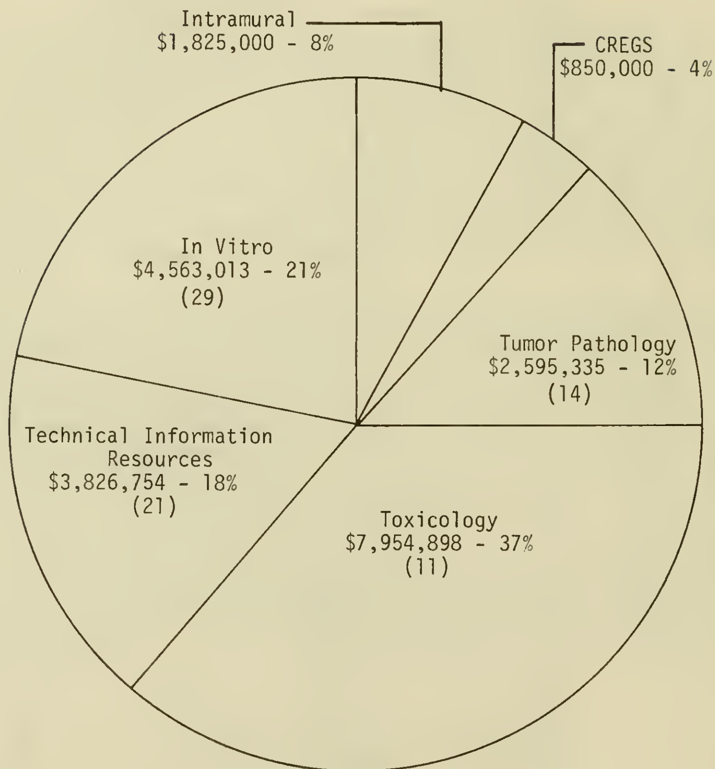
J. Ward, D.V.M., Ph.D. - Acting Chief
W. Edwards, Ph.D.[4]
K. Hoover, S.C.D.[5]
M. Levitt, M.D.
C. Lingeman, M.D.[3]
G. Reznik, D.V.M.[6]
S. Stinson, Ph.D.

[1]NIH Scientist Emeritus
[2]Guest Worker
[3]Part-time Employee
[4]Visiting Associate
[5]Staff Fellow
[6]Visiting Scientist

D. FISCAL INFORMATION

CARCINOGENESIS TESTING PROGRAM

OPERATIONAL BUDGET - $21,615,000
Fiscal Year 1978



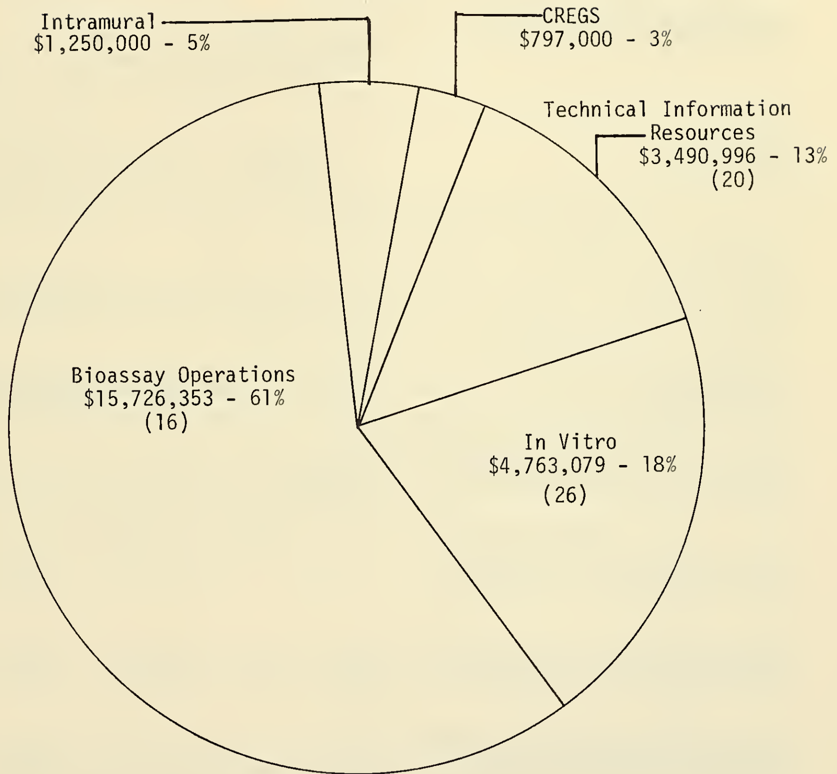
Note:

Dollars based on projected estimates and negotiated contracts as of May 4, 1978.

Numbers in parentheses below operating amounts represent active contracts in Program areas.

CARCINOGENESIS TESTING PROGRAM

OPERATIONAL BUDGET - $26,027,428
Fiscal Year 1977



Note:

Bioassay Operations encompasses several Program areas not identified separately, including Tumor Pathology and Toxicology Branches (officially defined July 26, 1977.)

Numbers in Parentheses below operating amounts represent active contracts in Program areas.

ANALYSIS OF CONTRACTS BY OPERATIONAL UNITS
IN THE CARCINOGENESIS TESTING PROGRAM

UNIT CONTRACT	TITLE
1. IN VITRO PROGRAM	
AMERICAN HEALTH FOUNDATION N01-CP-55705	Development of Detailed Methods and Protocols for Carcinogenic Screening Using Cell Culture Assays - Task V - Epithelial Cells
ARTHUR D. LITTLE, INC. N01-CP-55711	Development of Detailed Methods and Protocols for Carcinogenesis Screening Using Cell Culture Assays - Task II - BALB/c 3T3 Cells
BIOLABS, INC. N01-CP-45615	In Vitro Study of the Nature of the Interaction Between Chemical and Virus Carcinogens
CHILDREN'S HOSPITAL (at Akron) N01-CP-55706	Development of Detailed Methods and Protocols for Carcinogenesis Screening Using Cell Culture Assays - Task V
DOE - NCI INTERAGENCY AGREEMENT, OAK RIDGE NATIONAL LABORATORY Y01-CP-70222	Malignant Cell Transformation and Mutagenesis Induced by Carcinogenic Chemicals
DOE - NCI INTERAGENCY AGREEMENT, OAK RIDGE NATIONAL LABORATORY Y01-CP-50200	Chemical Carcinogenesis Cell Biology
DOE - NCI INTERAGENCY AGREEMENT, OAK RIDGE NATIONAL LABORATORY Y01-CP-70226	Support of a Committee on Chemical Environmental Mutagens
DOE - NCI INTERAGENCY AGREEMENT, OAK RIDGE NATIONAL LABORATORY Y01-CP-70227	In Vitro Transformation of Tumor Sensitive Epidermal Cells: A Bioassay and a Model for the Study of the Mechanism of Action of Tumor Initiators and Promoters
ILLINOIS, UNIVERSITY OF N01-CP-23303	Temperature Sensitive Mutants in In Vitro Carcinogenesis
INTERNATIONAL AGENCY FOR RESEARCH ON CANCER N01-CP-55630	The Significance of Experimental Carcinogenesis Data to Man

INVERESK RESEARCH INTERNATIONAL N01-CP-75858	Validation and Utilization of Microbial Mutagenesis Systems as Prescreens for Chemical Carcinogens
JOHN L. SMITH MEMORIAL FOR CANCER RESEARCH, PFIZER, INC. N01-CP-55703	Development of Detailed Methods and Protocols for Carcinogenesis Screening Using Cell Culture Assays - Task I and Task III - Fischer Rat Embryo Cells Infected with Rauscher Leukemia Virus and Fischer Rat Embryo Cells
LEO GOODWIN INSTITUTE FOR CANCER RESEARCH N01-CP-65804	Production of an In-Bred Syrian Hamster Colony
LITTON BIONETICS, INC. N01-CP-65853	Development and Validation of an In Vitro Mammalian Cell Mutagenesis System for Carcinogenesis Screening
LITTON BIONETICS, INC. N01-CP-65856	Validation and Utilization of Microbial Mutagenesis Systems as a Prescreen for Chemical Carcinogens
MICROBIOLOGICAL ASSOCIATES N01-CP-55670	Development of Detailed Methods and Protocols for Carcinogenesis Screening Using Cell Culture Assay - Task I - Fischer Rat Embryo Cells Infected with Rauscher Leukemia Virus
NCI - FREDERICK CANCER RESEARCH CENTER (LITTON BIONETICS, INC.) PROJECT #10 N01-CO-75380	In Vitro Carcinogenesis
NEW YORK MEDICAL COLLEGE N01-CP-65855	Validation and Utilization of Microbial Mutagenesis Systems as Prescreens for Chemical Carcinogens
NORTH CAROLINA, UNIVERSITY OF N01-CP-55707	Development of Detailed Methods and Protocols for Carcinogenesis Screening Using Cell Culture Assays - Task V - Epithelial Cells
OHIO STATE UNIVERSITY N01-CP-43276	In Vitro Study of the Nature of Interaction Between Chemical and Viral Carcinogens
SOUTHERN CALIFORNIA, UNIVERSITY OF N01-CP-65831	Carcinogenesis In Vitro: Initiation and Promotion

SRI INTERNATIONAL N01-CP-55701	Development of Detailed Methods and Protocols for Carcinogenic Screening Using Cell Culture Assays - Task IV - Hamster Host Mediated System
SRI INTERNATIONAL N01-CP-65854	Development and Validation of an In Vitro Mammalian Cell Mutagenesis System for Carcinogenesis Screening
SRI INTERNATIONAL N01-CP-65857	Validation and Utilization of Microbial Mutagenesis Systems as Prescreens for Chemical Carcinogens
SRI INTERNATIONAL N01-CP-75917	Detection and Identification of Mutagens in Human Body Fluids
WISCONSIN, UNIVERSITY OF N01-CP-85609	DNA Repair Studies in Cultured Hepatocytes

2. TECHNICAL INFORMATION RESOURCES

DEPARTMENT OF ENERGY - NCI INTERAGENCY AGREEMENT (NIEHS) Y01-CP-20203	Environmental Mutagen Information Center (EMIC)
EG&G/MASON RESEARCH INSTITUTE N01-CP-43257	Carcinogenesis Bioassay Data System (CBDS) Operational Support
EG&G/MASON RESEARCH INSTITUTE N01-CP-75957	Data and Information Resources
ENVIRO CONTROL, INC. N01-CP-75964	Data and Information Resources
FEIN-MARQUART ASSOCIATES, INC. N01-CP-75959	Data and Information Resources
FOOD & DRUG ADMINISTRATION - NCI INTERAGENCY AGREEMENT Y01-CP-70221	Development and Implementation of a Carcinogenesis Bioassay Management System
FRANKLIN INSTITUTE RESEARCH LABORATORIES N01-CP-75960	Data and Information Resources
FRANKLIN INSTITUTE RESEARCH LABORATORIES N01-CP-75885	Carcinogenesis Abstracts

ILLINOIS INSTITUTE OF TECHNOLOGY RESEARCH INSTITUTE N01-CP-75962	Data and Information Resources
INTERNATIONAL AGENCY FOR RESEARCH ON CANCER N01-CP-45608	Program on the Evaluation of Carcinogenic Risk of Chemicals to Humans
MITRE CORPORATION N01-CP-65863	Preparation of Carcinogen Bioassay Reports
PENNSYLVANIA, UNIVERSITY OF N01-CP-75958	Data and Information Resources
SRI INTERNATIONAL N01-CP-33285	A Research Program to Acquire and Analyze Information on Chemicals That Have Impact on Man and His Environment
SYSTEM SCIENCES, INC. N01-CP-75963	Data and Information Resources
TRACOR JITCO, INC. N01-CP-75961	Data and Information Resources

3. TOXICOLOGY

CALIFORNIA, UNIVERSITY OF SANTA CRUZ N01-CP-75816	Computer-Aided Prediction of Metabolites for Carcinogenicity Studies
CASE WESTERN RESERVE UNIVERSITY N01-CP-75927	Use of Physicochemical Parameters in Obtaining Structure-Activity Relationships with Potentially Cancer Related Endpoints
HOWARD UNIVERSITY N01-CP-33266	Chemical and Biological Investigation of Potential Carcinogens from Plants
JOHNS HOPKINS UNIVERSITY N01-CP-75929	The Use of Physicochemical Parameters in Obtaining Structure-Activity Relationships with Potentially Cancer Related Endpoints
NCI - FREDERICK CANCER RESEARCH CENTER (LITTON BIONETICS, INC.) PROJECT #8 N01-CP-75380	Bioassay Research
NEBRASKA, UNIVERSITY OF (EPPLY INSTITUTE) N01-CP-33278	Environmental Carcinogenesis Research in Bioassay

NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH FDA/HEW Y01-CP-80203	Workshop NCI/NCTR I/O Animals in Toxicology Testing*
PENNSYLVANIA STATE UNIVERSITY N01-CP-75926	The Use of Physicochemical Parameters in Obtaining Structure-Activity Relationships with Potentially Cancer Related Endpoints
SRI INTERNATIONAL N01-CP-75930	The Use of Physicochemical Parameters in Obtaining Structure-Activity Relationships with Potentially Cancer Related Endpoints
STANFORD UNIVERSITY MEDICAL CENTER N01-CP-75928	The Use of Physicochemical Parameters in Obtaining Structure-Activity Relationships with Potentially Cancer Related Endpoints
TEMPLE UNIVERSITY N01-CP-43271	UV Photocarcinogenesis: Effects of Depletion of Atmospheric Ozone
TRACOR-JITCO, INC. N01-CP-43350	Bioassay Prime Contract

Subcontractors:
- BATTELLE-COLUMBUS LABORATORIES
- DOW CHEMICAL COMPANY
- EG&G/MASON RESEARCH INSTITUTE
- GULF SOUTH RESEARCH INSTITUTE
- HAZELTON LABORATORIES OF AMERICA
- ILLINOIS INSTITUTE OF TECHNOLOGY RESEARCH INSTITUTE
- INDUSTRIAL BIO-TEST
- LITTON BIONETICS, INC.
- SOUTHERN RESEARCH INSTITUTE
- SRI INTERNATIONAL

Special Study Subcontractors:
- EXPERIMENTAL PATHOLOGY LABORATORIES
- METCALF & EDDY
- MIDWEST RESEARCH INSTITUTE
- PAPANICOLAOU CANCER RESEARCH INSTITUTE
- UNIVERSITY OF NEW ORLEANS
- WALDEN DIVISION OF ABCOR

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*First Annual NCTR/NCI Symposium/Workshop on The Use of Inbred and Outbred Animals in Toxicologic Testing was held on February 27 - March 1 at the Holiday Inn in North Little Rock, Arkansas. No contract narrative is provided.

4. TUMOR PATHOLOGY BRANCH

Contractor	Project
ALABAMA, UNIVERSITY OF (at Birmingham) N01-CM-67083	Rodent Disease Surveillance (Supplement to Division of Cancer Treatment contract)
AMERICAN HEALTH FOUNDATION N01-CP-75940	Long Term Studies of Prevention of Epithelial Cancer by Retinoids
CALIFORNIA, UNIVERSITY OF (at Davis) N01-CP-65845	Biology of Neoplastic Liver Lesions in Mice
CHARLES RIVER BREEDING LABORATORIES, INC. N01-CP-55651	Establishment of a Gnotobiote Originated Rodent Production Colony
EXPERIMENTAL PATHOLOGY LABORATORIES, INC. N01-CP-65731	Animal Pathology Support
HARLAN INDUSTRIES, INC. N01-CP-65737	Establishment of a Rodent Production Colony
ILLINOIS INSTITUTE OF TECHNOLOGY RESEARCH INSTITUTE N01-CP-75939	Long Term Studies of Prevention of Epithelial Cancer by Retinoids
JOHNS HOPKINS UNIVERSITY N01-CP-65768	Animal Pathology Support
MARYLAND, UNIVERSITY OF N01-CP-65752	The Biology of Neoplastic Liver Lesions in Mice
MICROBIOLOGICAL ASSOCIATES, INC. N01-CP-02199	Laboratory Service for Support in Carcinogenesis Bioassay and Related Activities
MIDDLESEX HOSPITAL MEDICAL SCHOOL N01-CP-75938	Long Term Studies of Prevention of Epithelial Cancer by Retinoids
NATIONAL ACADEMY OF SCIENCES/ INSTITUTE OF LABORATORY ANIMAL RESOURCES N01-CP-65805	Histologic Classification of Laboratory Animal Tumors
TEXAS, UNIVERSITY OF N01-CP-65846	The Biology of Neoplastic Liver Lesions in Mice
WISCONSIN, UNIVERSITY OF (at Madison) N01-CP-75905	Long Term Studies of Prevention of Epithelial Cancer by Retinoids

E. ADVISORY GROUPS AND CONSULTANTS

1. The Carcinogenesis Testing Program Review Group (CTPRG) is a committee of scientists who provide review of proposed activities for priority and relevance within the Carcinogenesis Testing Program. Membership includes the following individuals:

Carcinogenesis Testing Program Staff:

Dr. Richard A. Griesemer, Associate Director - Chairman
Ms. Joan Chase - Executive Secretary
Dr. Cipriano Cueto, Chief, Toxicology Branch
Dr. J. Fielding Douglas, Expert
Dr. Virginia Dunkel, Coordinator for In Vitro Program
Dr. Sidney Siegel, Acting Chief, Technical Information Resources Branch
Dr. Jerrold M. Ward[1], Acting Chief, Tumor Pathology Branch

Others:

Dr. Thomas Cameron, DCCP, NCI
Dr. Elizabeth Weisburger, CMT, CGR, DCCP, NCI

2. The Data Evaluation Group (DEG) is a group of scientists who (1) establish basic data requirements for specific types of reports, (2) establish criteria for data review and evaluation, (3) review and evaluate collated data as to its biological and statistical significance in the assessment of the possible carcinogenicity of a specific chemical to experimental animals, and (4) formulate recommendations as to data needs, limitation of interpretation and nature of report to be written. The Group consists of the following members:

NCI Staff:

Dr. Cipriano Cueto, Jr. - Chairman
Dr. Kenneth C. Chu
Dr. John J. Gart[2]
Dr. Dawn Goodman[3]
Dr. Richard A. Griesemer - Adviser
Dr. Morton Levitt
Dr. Harry A. Milman
Mr. Jun-mo Nam [2]
Dr. Thomas W. Orme
Dr. Hugh M. Pettigrew[2]
Dr. Robert A. Squire - consultant
Dr. Sherman Stinson
Dr. Robert E. Tarone[2]
Dr. Jerrold M. Ward
Dr. Carrie E. Whitmire

[1]Replaced Dr. Dawn Goodman who is no longer at NCI.
[2]Resigned as of March 10, 1978.
[3]No longer on staff.

Other:

Tracor Jitco, Inc.:	Dr. James Joiner Dr. Steve Olin - Adviser Mr. Wayne Reichardt - Executive Secretary Dr. Jane Robens
The Mitre Corporation:	Mr. William Belew[1] Dr. Mary Kornreich[1] Ms. Pam Walker

3. The Experimental Design Group (EDG) is a group of scientists who (1) establish basic objectives of the test and data elements required for each chemical considered; (2) establish specific experimental design to obtain the required data and provdie a written outline of the protocol recommended; and (3) maintain close interaction with the Data Evaluation Group, identify general objectives and problem areas in experimental design, and formulate recommendations. Members are as follows:

NCI Staff:

Dr. Cipriano Cueto, Jr. - Chairman
Dr. Thomas P. Cameron - Adviser
Dr. Kenneth C. Chu
Dr. J. Fielding Douglas
Dr. Dawn Goodman[1]
Dr. Richard A. Griesemer - Adviser
Dr. Morton Levitt
Dr. Harry A. Milman
Dr. Thomas W. Orme
Dr. Robert A. Squire - Adviser
Dr. Sherman Stinson
Dr. Jerrold M. Ward
Dr. Carrie E. Whitmire

Other:

NIEHS:	Dr. R.S. Chhabra Dr. Joyce Goldstein Dr. Eugene McConnell Dr. John M. Moore
Tracor Jitco, Inc.:	Dr. James Joiner Dr. Steve Olin - Executive Secretary Dr. Jane Robens

[1]No longer on staff.

4. The Chemical Selection Working Group (CSWG) is a group of scientists who nominate chemicals for testing in the Carcinogenesis Testing Program. The CSWG submits the names of the nominated chemicals to the Chemical Selection Subgroup of the Clearinghouse on Environmental Carcinogens for its advisory opinions. The Associate Director then gives the final approval on chemicals to be tested by the Program. Members of the group are as follows:

NCI Staff:

Dr. Herman F. Kraybill - Chairman
Dr. Elizabeth Weisburger - Co-Chairman
Dr. Thomas Cameron
Dr. Pierre Decoufle
Dr. J. Fielding Douglas - Executive Secretary
Dr. Virginia Dunkel
Dr. Lionel Poirier[1]
Dr. Sidney Siegel

Other:

U.S. Government Agencies:
Dr. Jack C. Dacre, U.S. Army
Mr. John M. Davitt, FDA
Dr. Harry W. Hays, USDA/ARS
Dr. Robert Hehir, CPSC
Dr. Bernard P. McNamara, DOA
Dr. Carl R. Morris, EPA
Dr. Lawrence Plumlee, EPA
Dr. Richard Rhoden, NIOSH
Dr. John Richardson, CDC
Dr. Murry Schulman, ERDA
Dr. Raymond Shapiro, NIEHS
Dr. Samuel I. Shibko, FDA
Dr. James Vail, OSHA

Tracor Jitco, Inc.:
Dr. C.W. Jameson - Executive Secretary
Ms. Linda Scheer
Ms. Pauline Wagner

5. The Carcinogenesis Program Scientific Review Committee provides expert peer review of proposals submitted to the Carcinogenesis Testing Program and the Carcinogenesis Research Program. Based on an evaluation of the scientific merit of the proposed work and on an evaluation of the technical competence of the proposed staff, the Committee provides recommendations concerning the award of contracts or other appropriate support instruments. Members are as follows:

[1]Resigned as of March 31, 1978

Dr. Robert E. Greenfield, Saint Vincent Hospital, Worcester, MA - Chairman

Dr. Virginia Dunkel, NCI - Co-Executive Secretary

Dr. Carl E. Smith - Co-Executive Secretary

Dr. Gerald L. Bartlett, Pennsylvania State University College of Medicine, Hershey, PA

Dr. Howard A. Bern, University of California, Berkeley, CA

Dr. Louis M. Fink, University of Colorado Medical School, Denver, CO

Dr. Sidney Green[1], Howard University College of Medicine, Washington, DC

Dr. Eliezer Huberman[1], Oak Ridge National Laboratory, Oak Ridge, TN

Dr. Phillip Issenberg, Eppley Institute for Research in Cancer, University of Nebraska, Omaha, NB

Dr. Albert M. Jonas[1], Yale University School of Medicine, New Haven, CT

Dr. M. Edward Kaighn, Pasadena Foundation for Medical Research, Pasadena, CA

Dr. Louise S. Lombard, Argonne National Laboratory, Argonne, IL

Dr. Prabhakar D. Lotlikar, Fels Research Institute, Temple University School of Medicine, Philadelphia, PA

Dr. Danuta Malejka-Giganti, Veterans Administration Hospital, Minneapolis, MN

Dr. Yvonne Connolly Martin[1], Abbott Laboratories, North Chicago, IL

Dr. Ian C. Munro[1], Health and Welfare, Ottawa, Ontario, Canada

Dr. Roy Ritts, Mayo Graduate School of Medicine, Rochester, MN

Dr. Evelyn M. Rivera, Michigan State University, East Lansing, MI

Dr. James A. Swenberg[1], Upjohn Company, Kalamazoo, MI

Other consultants who have been utilized by the Program throughout this fiscal year as contract site visitors, reviewers, individual technical advisors, or lecturers are as follows:

Dr. Wallace D. Armstrong, University of Minnesota, Minneapolis, MN

Dr. Edward C. DeFabo, EPA, Washington, DC

[1]Appointments pending final clearances.

Dr. John H. Epstein, University of California Medical Center, San Francisco, CA

Dr. Al Forziati, EPA, Washington, DC

Dr. Sidney Green, Howard University College of Medicine, Washington, DC

Dr. Thomas Griffin, Tracor Jitco, Inc., Rockville, MD

Dr. Sara Henry, FDA, Washington, DC

Dr. Harold C. Hodge, University of California, San Francisco, CA

Dr. Margaret Kripke, FCRC, Fort Detrick, Frederick, MD

Dr. Prabhakar Lotlikar, Fels Research Institute, Temple University School of Medicine, Philadelphia, PA

Dr. Veronica Maher, Michigan State University, East Lansing, MI

Dr. Hans W.J. Marquardt, Memorial Sloan-Kettering Cancer Center, New York, NY

Dr. James L. McQueen, New Hudson, MI

Dr. Steve O'Brien, NCI, NIH, Bethesda, MD

Colonel William H. Pryor, National Naval Medical Center, Bethesda, MD

Dr. Herbert S. Rosenkranz, New York Medical College, Valhalla, NY

Dr. Richard Setlow, Brookhaven National Laboratory, Upton, NY

Dr. James H. Shaw, Harvard School of Dental Medicine, Boston, MA

Dr. Andrew Sivak, A.D. Little, Inc., Cambridge, MA

Dr. John D. Spikes, University of Utah, Salt Lake City, UT

Dr. Donald R. Taves, University of Rochester Medical Center, Rochester, NY

Dr. Snorri Thorgeirsson, NCI, NIH, Bethesda, MD

Dr. I. Bernard Weinstein, Institute of Cancer Research, Columbia, University, New York, NY

Dr. Gary M. Whitford, Medical College of Georgia, Augusta, GA

Dr. Robert A. Whitney, Jr., DRS, NIH

Dr. Gary Williams, Naylor/Dana Institute, American Health Foundation, Valhalla, NY

III. SCIENTIFIC HIGHLIGHTS

A. IN VITRO CARCINOGENESIS PROGRAM

October 1, 1977 through September 30, 1978

The major goals of the In Vitro Program are 1) to establish a battery or matrix of procedures which can be used as an effective pre-screen for establishing priorities for in-depth carcinogenicity testing, 2) to define the utility of in vitro test procedures for assisting in the evaluation of marginal data on carcinogenicity or mutagenicity derived from animal experiments, and 3) to provide research tools for investigation of the mechanism of action of chemical carcinogens, including identification of molecular targets and the steps in carcinogenic process.

In the past few years considerable research has been carried out to develop in vitro methods which could be used to determine the carcinogenic potential of chemical compounds. These in vitro assays can be broadly divided into three major categories, namely, those in which there is 1) induction of neoplastic transformation of mammalian cells in culture, 2) mutagenic or chromosomal changes in various microorganisms or mammalian cells and 3) interactions between the chemical carcinogens and target macromolecules such as DNA. Assays from each of these categories are currently in the process of being defined and evaluated in terms of usefulness, reproducibility and correlation of response with chemicals of known in vivo carcinogenic activity.

In considering the overall validation of the short-term in vitro assays, the general approach has been directed to:

1. Development, definition and standardization of methodology which could be used for routine testing

2. Determination of the reproducibility of the defined protocol

3. Evaluation of a series of coded carcinogens and non-carcinogens from different chemical classes, and

4. Determination of the inter-laboratory reproducibility of the methodogy.

The data accumulated from these studies will provide a base of information for each of the model systems so that a final correlation of the in vitro test results can be made with those obtained in in vivo studies. In addition, the data from these studies will also provide the means to assess the relative merits of the various in vitro assays.

The initial effort with all the mammalian cell transformation assays being evaluated was to define the methodology with consideration for 1) selection and characterization of susceptible target cell populations; 2) determination

of the ability of the cells to activate procarcinogens to their ultimate reactive form; 3) evaluation of endpoints other than morphology which would be valid indicators of the carcinogenic potential of the chemicals tested, and 4) incorporation of exogenous activation systems for those procarcinogens requiring activation through metabolic pathways not operational within the cells in culture. Using this approach, testing protocols have been defined for those assays using BALB/c 3T3 cells (Arthur D. Little, Inc. - NO1-CP-55711); Fischer rat embryo cells infected with Rauscher leukemia virus (Microbiological Associates, Incorporated, NO1-CP-55670); and Pfizer, Incorporated, John L. Smith Memorial for Cancer Research Center - NO1-CP-55703) and hamster embryo cells (Frederick Cancer Research Center - NO1-CO-25423).

Studies with coded chemicals are in progress with all these assays. Two other cell systems, namely, those using uninfected Fischer rat embryo cells (Pfizer, Incorporated, John L. Smith Memorial for Cancer Research NO1-CP-55703) and hamster embryo cells exposed to the chemical transplacentally (SRI-International NO1-CP-55701) do not appear to be of value for routine screening and further studies with these assays is being terminated.

In addition to these studies with fibroblastic cells, effort has also been directed to the development of epithelial cell systems which could be used for screening chemicals. Since epithelial cells do not exhibit morphological changes in the cells after treatment with chemicals such as those seen with fibroblasts, the utilization of other endpoints which might be indicative of carcinogenic event are being explored. These include DNA damage and repair, specific enzymatic changes, and cultural characteristics. DNA repair measured by autoradiography has been induced in primary cultures of hepatocytes from Fischer rats by a large series of activation-dependent carcinogens from different classes. This method developed by the American Health Foundation (NO1-CP-55705) is currently being used to test coded chemicals. This laboratory has also demonstrated that HGPRT deficient mutants can be induced in long-term cultures of rat epithelial cells and that a feeder layer of primary rat hepatocytes can provide the means for activation of procarcinogens. Other endpoints for rapid screening in cultured liver epithelial cells which are being assessed include increased saturation density and increased colony forming ability. These appear to hold promise and are being further evaluated (University of North Carolina - NO1-CP-55707). Although the correlation between mutagenicity and carcinogenicity has more than adequately been demonstrated with the Salmonella typhimurium strains developed by Dr. Bruce Ames a collaborative study was undertaken to determine the interlaboratory reproducibility of microbial mutagenicity assays as well as DNA repair assays. The four laboratories participating in this study are Inveresk Research International (NO1-CP-75858), Litton Bionetics, Incorporated (NO1-CP-65856), New York Medical College (NO1-CP-65855) and SRI International (NO1-CP-65857). Testing of approximately 50 carcinogens and non-carcinogens has been completed and chemicals tested in the NCI Bioassay Program are scheduled for evaluation. All chemicals are being tested both with and without metabolic activation in mutagenicity assays using Salmonella typhimurium strains TA-1535, 1537, 1538, 98 and 100 and Escherichia coli strain WP2 uvrA$^-$ and in the DNA repair assay

which uses Escherichia coli strains W3110 polA^+ and p3478/polA^-. The metabolic activation systems used in this study are being derived from the livers of both uninduced and arochlor 1254-induced Fischer 344 rats, $B_6C_3F_1$ mice (C57Bl6 x C3H[MTV-$_{F-1}$] and Syrian hamsters. Since the amount of information generated in these contracts is extensive, a data base has been established in order to facilitate handling and analysis of the results. This system is now in its final stages of testing to ensure that the data can be properly captured and that the information can be retrieved in a meaningful way.

Developmental and evaluation studies are also with the mammalian mutagenesis assay using induction of $TK^-/^-$ mutants in L5178Y ($TK^+/^-$) mouse lymphoma cells. This work is being carried out by Litton Bionetics, Incorporated (N01-CP-65853) and SRI International (N01-CP-65854). Studies were initially carried out to ensure that the procedure could be established as previously published and to determine whether there was a need for modifications in the test procedure. A protocol has been defined which is being used by both laboratories to test a series of 16 reference chemicals. With completion of testing with these compounds studies with coded chemicals will be initiated. The chemicals used for the latter part of the evaluation will be the same as those tested in microbial assays.

Although initial emphasis within the In Vitro Program has been on development and validation of various short-term assays, there is a series of contracts which support research on the development of in vitro carcinogenesis model systems and mechanisms of action. These studies are as follows: 1) determination of the process by which chemical carcinogens promote viral oncogenesis and whether oncogenic and non-oncogenic viruses may promote transformation by known chemical carcinogens in the same way (Biolabs, Inc. - N01-CP-45615); 2) comparison of metabolism of carcinogens by human and rodent tissues and reactivity of carcinogens and their metabolites in mutagenesis and cell transformation assays (International Agency for Research on Cancer - (N01-CP-55630); 3) development of both an initiation/promotion system and an assay of syn-carcinogenesis for combinations of weak carcinogens in the C_3H 10T 1/2 cell line (University of Southern California - N01-CP-65831); 4) determination of the characteristics and responsiveness of the BHK21 hamster cell system to chemical carcinogens (University of Illinois - N01-CP-23303); 5) evaluation of human cell culture systems for in vitro carcinogenesis studies and the role of hormones as inhibitors or promoters and viruses as promoters of carcinogenesis (Ohio State University - N01-CP-43276); 6) development of transformation models using epidermal cells from tumor sensitive and tumor resistant mice so that an evaluation can be made of any possible differences in response of the cells to carcinogens (ERDA-Oak Ridge National Laboratory (N01-CP-70227); 7) studies on the mechanisms of metal carcinogenesis in vitro and co-transforming potential of metals when given with carcinogen chemicals from different classes (ERDA - Oak Ridge National Laboratory - N01-YO-70222). and 8) development of methods using microbial mutagenicity assays for screening human urine for mutagenic metabolites (SRI International - N01-CP-75917).

As support for those contractors using hamsters and hamster embryo cells, the Leo Goodwin Institute for Cancer Research (N01-CP-65804) maintains a breeding colony and supplies Syrian hamsters.

IV. CONTRACT NARRATIVES

A. IN VITRO PROGRAM

October 1, 1977 through September 30, 1978

AMERICAN HEALTH FOUNDATION (N01-CP-55705)

Title: Development of Detailed Methods and Protocols for Carcinogen Screening Using Cell Culture Assays - Task V - Epithelial Cells

Contractor's Project Director: Dr. Gary M. Williams

Project Officer (NCI): Dr. Virginia C. Dunkel

Objectives: To determine the response of epithelial cell cultures to carcinogens, procarcinogens and noncarcinogenic analogues in order to develop methods and procedures for screening of chemical carcinogens.

Major Findings: Three approaches are being studied for assaying the effect of carcinogens on cultured rat liver epithelial cells as follows: (1) the induction of DNA repair by carcinogens in primary cultures of rat liver hepatocytes; (2) the induction of hypoxanthineguanine phosphoribosyl transferase deficient mutants in long-term cultures of rat liver epithelial cells and (3) the induction of phenotypic transformation in long-term cultures of rat liver epithelial cells.

DNA repair measured autoradiographically was induced in the primary cultures of hepatocytes by three activation-independent carcinogens and, therefore, subsequent testing was concentrated on activation-dependent carcinogens. DNA repair was elicited by 19 out of 20 activation-dependent carcinogens from 5 structurally different classes including mycotoxins, polycyclic aromatic hydrocarbons, aromatic amines, azo dyes, and nitrosamines. Nine out of 10 structually related noncarcinogenic analogues.

Mutants in rat liver epithelial cell lines have been induced by exposure to the activation-dependent carcinogens, aflatoxin B_1, N-2-fluorenylacetamide, 7,12-dimethylbenz(a)anthracene and 3'methyl-4-dimethylaminoazobenzene. These studies provide evidence of significant ability for carcinogen activation by the rat liver epithelial lines. Nevertheless, a system was devised for employing the high metabolic capability apparent in the primary cultures of hepatocytes to provide activation of carcinogens to metabolites mutagenic to liver epithelial lines. In this system, primary hepatocytes were co-cultivated with liver epithelial cells as the target cells. Hepatocyte-mediated mutagenesis resulted from exposures to levels of activation-dependent carcinogens that did not mutate the epithelial cells by themselves.

Eleven different markers associated with transformation in other systems have been examined in the rat liver epithelial lines. Of these, growth in soft agar,

high uptake of 2-deoxyglucose, and activity of gamma-glutamyl transpedtidase correlated with tumorigenicity of the lines. The induction of these markers following exposure to carcinogens is now being studied.

Significance to Biomedical Research and the Program of the Institute: The major goal of this project is to develop rapid and reproducible screening assays for chemical carcinogens in epithelial cultures. The induction of DNA repair in primary cultures of hepatocytes and the induction of mutagenesis in the long-term rat liver epithelial cultures both in the lines alone and mediated by co-cultivated primary hepatocytes appear to provide suitable screening systems. Thus, it may be possible to use these systems for evaluating the potential carcinogenicity of chemicals and establishing priorities for bioassay.

Proposed Course: (1) To further evaluate the sensitivity of the induction of DNA repair and of mutagenesis as screening systems; (2) To evaluate the induction of phenotypic transformation in the long-term cell cultures as a screening system, and (3) To develop additional assays for the carcinogenic effects of chemicals on epithelial cell cultures.

Date Contract Initiated: June 30, 1975

Current Annual Level: $161,949

ARTHUR D. LITTLE, INCORPORATED (N01-CP-55711)

Title: Development of Detailed Methods and Protocols for Carcinogenesis Screening Using Cell Culture Assays - Task II - BALB/c3T3 Cells

Contractor's Project Director: Dr. Andrew Sivak

Project Officer (NCI): Dr. Virginia C. Dunkel

Objectives: To develop and standardize methods for performing in vitro transformation assays with BALB/c3T3 cells and early passage Fischer rat embryo cells using chemical carcinogens as the transforming agents.

Major Findings: Validation studies with 13 known chemical compounds using clone 1-13 (Kakunaga) of BALB/c3T3, showed that direct-acting and polycyclic aromatic hydrocarbon carcinogens gave a positive response by producing Type III foci in a dose related fashion. Compounds not active in vivo as carcinogens showed no activity in the cell culture assay. Dimtheylnitrosamine was not active indicating that the target cells did not obtain the necessary enzymes for activation of this class of chemicals. Various factors influencing the transformation assay were examined including serum source, serum amount, type of medium, plating density of target cells, passage, exposure time for polycyclic aromatic hydrocarbons, and assay duration. Transformed foci yielded cell populations that grew increasingly well in soft agar with repeated passage and that produced fibrosarcomas in weanling BALB/c mice.

Results from the testing of 18 coded samples revealed in general a good correlation with in vivo carcinogenic activity of the chemicals tested. Aromatic amines and nitrosamines showed no transforming activity on cells of clone 1-13.

Studies with S-9 fractions from rat liver revealed that toxicity of these fractions was a severe problem in designing exogenous activation experiments to allow the detection of chemicals not metabolized by the target cells.

Significance of Biomedical Research and the Program of the Institute: The carcinogenic risk to man from chemical agents now present in the environment or being introduced anew is largely unknown. The task of testing these materials by the classic chronic animal exposure protocols is formidable and prohibitive with respect to resources. While the short-term screening tests for mutagenicity with microorganisms are more reasonable in cost, they are at best only indicative of some genomic alterations and do not adequately reflect the process of carcinogenesis. The cell culture systems under study should provide standardized procedures to examine chemicals for carcinogenicity in short-term systems of modest cost that have genuine biological relevance to the process of carcinogenesis. Further, these cell culture systems should allow a more detailed explanation of the sequential cellular processes occurring in the development of neoplastic disease.

Proposed Course: The overall objective is to develop and validate a reliable and reproducible assay system for the demonstration of neoplastic transformation in a cell culture system using BALB/c3T3 mouse embryo fibroblasts. The specific accomplishments proposed for the next contract interval are: (1) Continue identification of factors which influence the assay and determine procedures for their standardization. (2) Complete assay of coded samples supplied by the National Cancer Institute. (3) Refine and implement means for activation of chemicals requiring metabolism by enzymatic processes either not present or present only at the insufficient levels in target cells. (4) Determine whether initiation-promotion protocols can substantially enhance the sensitivity of the assay and/or identify initiators not capable of inducing transformation alone.

Date Contract Initiated: June 30, 1975

Current Annual Level: $228,412

BIOLABS, INCORPORATED (N01-CP-45615)

Title: In Vitro Study of the Nature of the Interaction Between Chemical and Viral Carcinogens

Contractor's Project Director: Dr. Bruce C. Casto

Project Officer (NCI): Dr. Joseph A. DiPaolo

Objectives: The specific objective of this contract is to study the process whereby chemical carcinogens promote viral oncogenesis and to determine if oncogenic and non-oncogenic viruses may similarly promote carcinogenesis by known chemical carcinogens.

Major Findings: This study has established that treatment of cells with chemical carcinogens and mutagens will increase the frequency of viral transformation in vitro. Approximately 200 chemicals including polycyclic hydrocarbons, aromatic amines, alkylating agents, inorganic metal salts, drugs, fuel or food additives, pesticides, herbicides, organic solvents and other industrial chemicals have been or are being tested in hamster cells for toxicity and their capacity to enhance viral transformation, induce DNA strand breaks, stimulate unscheduled DNA (repair) synthesis, induce mutations, and transform cells in vitro.

All of the chemicals tested that are known to be, or suspect, carcinogens in man or animals will enhance viral transformation with the exception of certain chemicals that may not be metabolized by hamster cells in vitro including; red dye #2, chloroform, trichloroethylene, N-nitrosodiethylamine, N-nitrosodimethylamine, urethane, thiourea, and ethylenethiourea. When incubated with a hamster liver S-9 activation system, N-nitrosodimethylamine and urethane will enhance. Several chemicals such as manganous chloride and 2-chloro-1,3-butadiene, that do not cause enhancement when preincubated with confluent cells, are positive when added post-virus inoculation and cell transfer. Forty-two of the 50 chemicals produced in billions of lbs./yr. have been tested in the viral enhancement assay. Propylene oxide, vinyl acetate, and ethylene dichloride are positive for enhancement in hamster cells. In addition, inorganic metal salts have been tested with positve enhancement obtained with metals known to be carcinogenic or mutagenic in other assay systems.

Chemicals shown to enhance viral transformation may or may not cause DNA breaks and repair synthesis; however, all chemicals that break DNA or induce repair will enhance. Approximately 77 chemicals have been examined for their capacity to induce DNA strand breakage. Forty of 55 known carcinogens induced DNA fragmentation when analyzed on alkaline sucrose gradients, whereas 17 of 18 assumed negative carcinogens were also negative for DNA breakage. Of the chemicals assayed for DNA repair in Syrian hamster embryo cells, only 14 of 50 (28%) known carcinogens stimulated DNA repair synthesis; all non-carcinogens (20 tested) were negative.

Enhancement of viral transformation by nickel sulfate and methyl methanesulfonate is associated with an increased integration of viral DNA into cell DNA as demonstrated by cRNA-DNA hybridization studies. Persistence of enhancement following chemical treatment with MMS, MNNG, Ac-AAF, B(a)P or DMBA appeared to be related to the rate of repair of DNA damage.

Chemicals that stimulate the frequency of viral transformation in Syrian hamster embryo cells are being tested in the same cell system for carcinogenic transformation without added virus. In this focus assay model, 70 chemicals have been tested. Fifty of 55 known carcinogens caused transformation of

hamster embryo cells, whereas none of the non-carcinogens or solvent controls yielded transformed foci. Cell lines derived from transformed foci have been characterized according to their: (1) tumorigenicity in animals; (2) capacity to clone in soft agar, concanavalin A, heparin, dextran sulfate, DEAE dextran or reduced serum; and (3) response to normal cell constitutents. Those properties more closely associated with tumorigenicity were the ability to clone in soft agar, resistance to dextran sulfate or heparin, and cloning efficiency in 1% serum.

Experimental models have been developed to study the interaction between viruses and chemical carcinogens in vivo. Tumors induced in hamsters by intradermal inoculation of 3-methylcholanthrene, followed by certain adeno- or herpes viruses at the same site, have shown decreased latent periods in contrast to tumors induced by 3-methylcholanthrene alone.

Significance to Biomedical Research and the Program of the Institute: In view of the various hypotheses concerning chemicals, viruses, and cancer, and due to the inherent difficulties in studies in the intact animal, it is advantageous to study the various interactions in a controlled in vitro system. This would establish not only the influence of oncogenic and non-oncogenic viruses in promoting chemical carcinogenesis, but also the role of chemicals in promoting viral carcinogenesis. This sensitive quantitative assay may be used to detect potential carcinogenic or mutagenic chemicals in the environment.

Proposed Course: Diverse chemicals that are important to the human environment, including food additives, pesticides, herbicides, and other industrial chemicals will continue to be examined for their ability to enhance viral transformation. The nature of the enhancement of viral transformation following chemical treatment will be the subject of future studies, especially the role of DNA repair and breaks. Factors known to influence the toxicity and carcinogenicity of certain chemicals will be studied to determine their influence on the enhancement of quantitative transformation of cells by virus. Other types of cells and viruses will be employed to determine if the phenomenon of enhancement occurs in other systems. The influence of viruses on the frequency of transformation of hamster cells by chemicals will be explored using a quantitative, focus assay that does not require the use of feeder cells. Methods developed for chemical transformation of hamster cells will be used to investigate the sensitivity of other cell systems for in vitro transformation. Attempts will be made to determine the relationship between enhancement of viral transformation, DNA repair, DNA breaks, and chemical transformation of Syrian hamster cells.

Date Contract Initiated: June 9, 1971

Current Annual Level: $332,991

THE CHILDREN'S HOSPITAL MEDICAL CENTER OF AKRON (N01-CP-55706)

Title: Development of Detailed Methods and Protocols for Carcinogenesis Screening Using Cell Culture Assays - Task V - Epithelial Cells

Contractor's Project Directors: Dr. Howard J. Igel
Dr. Robert Lake

Project Officer (NCI): Dr. Virginia C. Dunkel

Objectives: To determine the response of epithelial cell cultures derived from human and rabbit skin to carcinogens, procarcinogens and non-carcinogenic analogues in order to develop methods and procedures for screening chemical carcinogens.

Major Findings: Methods for growth of adult diploid rabbit skin and human foreskin epithelial cells on a pigskin dermis substrate, irradiated fibroblast feeder layers and conventional plasticware have been developed and optimized. In addition, pools of human and rabbit skin mince have been cryopreserved and quantitatively recovered for subsequent epithelial culture.

A battery of long term biological markers including aneuploidy, mass and clonal morphology, liquid and soft agarose plating efficiency and in some cases tumorigenicity has been evaluated in rabbit and human epithelial cell cultures as predictors of carcinogenicity for a group of 17 chemicals including alkylating agents, polycyclic hydrocarbons and nitrosamines. A battery of short-term endpoints which includes toxicity, DNA repair synthesis (UDS) and DNA damage has also been developed as endpoints for the in vitro assessment of chemical carcinogens. Comparison of the predictive value of the long-term markers with the predictive value of toxicity and UDS in human and rabbit epithelial cell cultures clearly showed the superiority of the short-term markers.

In the rabbit model system certain polycyclic hydrocarbons were selectively toxic for epithelial cells. The data show that those polycyclic hydrocarbons which are known carcinogens exhibit a selective toxicity whereas non-carcinogenic polycyclic hydrocarbons tested did not. However, for other classes of carcinogens, selective epithelial cell toxicity had no correlation with carcinogenic potential. All compounds tested and found to be toxic to either the fibroblasts or epithelial cells present in the cultures at a concentration below 12.5 ug/ml were carcinogens. At the other end of the spectrum, several known carcinogens including DB(a,h)A, DCC, MAMA and DMN showed no toxicity to either cell type over the range of doses tested. In addition to this toxicity data, the 17 chemicals were assayed for induction of UDS. In general, there was excellent correlation between the known carcinogenicity of the compounds and the induction of UDS. Induction of >1000 dpm above background DNA synythesis, at some dose over the range tested and appearing in dose related fashion, was employed as the criterion for significant UDS. The carcinogenicity of 15 of the 17 compounds was accurately predicted by this endpoint.

Chemicals examined in the rabbit system were also examined in human skin cultures. All ultimate carcinogens tested induced UDS. The pro-carcinogenic polycyclic hydrocarbons, MCA, B(a)P, DMBA, and DB(a,h)A, also cause detectable UDS. In contrast, 2-AAF, TRIS, DMN, and 4AAB did not elicit a UDS response in human skin cultures.

Significance to Biomedical Research and the Program of the Institute: Human skin epithelial cell cultures have been effectively used in an in vitro carcinogenesis assay using UDS as an endpoint. While the validity of this assay as a general screen remains to be established, initial studies indicate that this assay will prove to be a relevant one for assessing the potential of xenobiotic chemicals as human skin carcinogens.

Proposed Course: Validate the use of a human cell assay based on repair of DNA damage for carcinogen screening using blind coded samples. Establish the reproducibility of the assay using a standardized pool of frozen skin cells. Assay a large number of chemicals including carcinogens and noncarcinogenic analogues for which adequate in vivo data exists to further define the predictive value of the assay. Develop additional short-term in vitro end points to be used in this assay with or independently of UDS.

Date Contract Initiated: June 30, 1975

Current Annual Level: $240,161

DEPARTMENT OF ENERGY-NCI INTERAGENCY AGREEMENT (OAK RIDGE NATIONAL LABORATORY (Y01-CP-70227)

Title: In Vitro Transformation of Tumor Sensitive Epidermal Cells: A Bioassay and a Model for the Study of the Mechanism of Action of Tumor Initiators and Promoters

Contractor's Project Director: Dr. Thomas J. Slaga

Project Officer (NCI): Dr. Virginia C. Dunkel

Objectives: To develop a reliable and quantitative in vitro transformation system using mouse epidermal cells from tumor sensitive mice, and to compare carcinogen induced changes in studies involving these epidermal cells with those in epidermal cells from tumor resistant mice, and to study the role of polyamines in tumor promotion and determine the specific localization of 12-O-tetradecanoyl-phorbol-13-acetate in epidermal cells.

Major Findings: Transformation studies using the epidermal cells from tumor sensitive mice are in progress. Preliminary studies suggest that epidermal cells from tumor sensitive mice cultured at 31°C in Waymouth's 752/1 supplemented with 10% fetal calf serum and with the addition of insulin (10 ug/ml), hydrocortisone (0.1 ug/ml) and epidermal growth factor (10 ng/ml may provide the proper conditions to obtain a quantitative and reliable transformation system.

Significance to Biomedical Research and the Program of the Institute: Since the majority of human tumors are epithelial in origin, the need exists to have reliable in vitro systems using epithelial systems in order to study the interactions between carcinogens and target cell macromolecules. It is possible that acquisition of basic information could lead to the development of means to inhibit or interrupt the carcinogenic process and thus reduce the incidence of cancer.

Proposed Course: To continue the studies as specified under objective.

Date contract initiated: September 30, 1977

Current Annual Level: $200,900

DEPARTMENT OF ENERGY-NCI INTERAGENCY AGREEMENT (OAK RIDGE NATIONAL LABORATORY (Y01-CP-70222)

Title: Malignant Cell Transformation and Mutagenesis Induced by Carcinogenic Chemicals

Contractor's Project Director: Dr. Eliezer Huberman

Project Officer (NCI): Dr. Virginia C. Dunkel

Objectives: The objective of this project is to study the cellular mechanisms of metal carcinogenesis in mammalian cells in culture. Specifically the study is concerned with the following: 1) susceptibility of mammalian cells to the cytotoxic effect of carcinogenic metals; 2) induction of malignant cell transformation directly by the carcinogenic metals, or indirectly by testing their effect as co-transforming agents with other classes of chemical carcinogens; 3) induction of mutations at different genetic loci. Mutagenesis by the metals is being tested directly or indirectly by determining their effect on mutagenesis induced by other classes of carcinogens such as nitrosamides, and polycyclic hydrocarbons.

Major Findings: Cell susceptibility of the Chinese hamster V79 cells to the cytotoxic effect of 8 different potentially carcinogenic metals was established. Cell susceptibility was determined by the ability of the V79 cells to form cell colonies after pretreatment for 4 and 24 hours with different metal concentrations. The metals could be divided into 4 groups in respect to the Do, a dose that results in a survival fraction of 37% colony forming cells. One group included $CaCrO_4$ and $CdCl_2$ with a Do of 5 $\pm$ 0.5 μM and 13 $\pm$ 0.5 μM after 24 or 4 hours of treatment, respectively. A second group included only Na_2HAsO_4 with a Do of about 0.15 mM after 4 hours treatment. A third group included $CoCl_2$ and $NiCl_2$ with a Do of 0.15 $\pm$ 0.05 mM and 0.62 $\pm$ 0.2 mM after 24 and 4 hours treatments, respectively. The least cytotoxic were $BeCl_2$ and $MnCl_2$ which gave a Do of 2.6 $\pm$ 1.8 mM and 9 $\pm$ 3 mM for the 24 or 4 hours treatments, respectively. These doses were used as the effective doses for mutagenesis.

Mutagenesis by the carcinogenic metals $BeCl_2$, $NiCl_2$, and $CaCrO_4$, was tested in the V79 cells using resistance to ouabain and 6-thioguanine as the genetic markers. These markers affect the surface membrane Na^+/K^+-ATPase and the hypoxanthine and guanine phosphoribosyl transferase (HGPRT), respectively. The 3 tested carcinogenic metals as well as two non-carcinogenic and non-cytotoxic metals Mg^{+2} and Ca^{+2} did not induce ouabain resistant mutants. Mutagenesis was obtained with the HGPRT marker after treatment with the carcinogenic metals. However, the degree of mutant induction was only up to five fold higher than the untreated controls.

In preliminary experiments on the co-mutagenic effects of the metals, it was found that Be^{+2} inhibited the mutagenic effect of a diol-epoxide of benzo(a) pyrene and enhanced the mutagenic effect of another carcinogen nitrosoguanidine. These results suggest that different metals may have acted differently as co-mutagens when combined with different classes of chemical carcinogens.

Cell transformation was tested in the golden syrian hamster embryo assay. Morphological transformation was determined after treatment of secondary golden syrian hamster embryo cells seeded for colony formation on an X-irradiated feeder layer of rat embryo cells. Transformed colonies were obtained after treatment with 0.1 - 3 μM of $CaCrO_4$ with a frequency of up to about 1%. No such morphological transformed colonies could be detected after treatment with 0.1 - 5 mM $BeCl_2$. Treatment of sparse cultures (5 x 10^5 cells per 50mm Petri dish) with these metals and their serial passages for three months resulted in the appearance of transformed cells with a high cloning efficiency (40-80%) and an increased saturation density (6 - 10 x 10^6 cells per 60 mm Petri dish).

Significance to Biomedical Research and the Program of the Institute: Metals are common contaminants in our environment as a result of coal combustion, as by-products of industries and as ingredients of human and veterinary medicines. Epidemiological studies have implicated some metals as being carcinogenic to humans. Determination of mechanisms in metal carcinogenesis could therefore provide information about inter-relationships between metals and other carcinogens and aid in assessing and modifying environmental exposure to combinations of compounds.

Proposed Course: In addition to $BeCl_2$, $NiCl_2$ and $CaCrO_4$, we shall include in our studies on the cellular effects of metals the following carcinogenic metals; $CdCl_2$, Na_2HAsO_4, $CoCl_2$, $MnCl_2$, and $ZnCl_2$. Mutagenesis will be determined in the Chinese hamster V79 cells as well as in human cells using 6-thioguanine and temperature resistance as the genetic markers. Transformation will be determined in the golden syrian hamster cells using both the colony assay as well as selection of transformed cells by a serial passage of treated cells.

Date Contract Initiated: May 9, 1977

Current Annual Funding Level: $242,000

DEPARTMENT OF ENERGY-NCI INTERAGENCY AGREEMENT (OAK RIDGE NATIONAL LABORATORY (Y01-CP-50200)

Title: Chemical Carcinogenesis Cell Biology

Contractor's Project Director: Dr. Raymond W. Tennant

Project Officer (NCI): Dr. Joseph A. DiPaolo

Objectives: The objective of this project is to independently evaluate the transplacental assay system developed by DiPaolo and co-workers (1973) for identifying the oncogenic potential of chemicals.

Major Findings: A protocol, based on colony morphology, was established and used in 1975-76 to test chemicals of known and unknown carcinogenic activity in the host-mediated in vivo-in vitro assay. However, in 1976-77, the development of problems in cell culture, primarily related to the health status of animals, has required a change in the criteria used to assess transformation. Progress has been made in applying the focus assay method. Continued development of this technique in the host-mediated assay system may lead to a more defined and reliable estimate for determining the oncogenic potential of chemicals.

Hamsters from both commercial and private colonies have been tested for health status and other factors in the host-mediated assay. Currently, animals obtained from Engle Farm Laboratory provide the most reliable source. Considerable effort was also directed at testing various culture media used in performing the colony assay. Dulbecco's modified medium marketed by KC Biological has provided the best growth support for hamster cells if supplemented with 10% unheated Reheis fetal calf serum (FCS). A cloning efficiency of 25% has been routinely obtained without any feeder layer; however, the cloning efficiency of the hamster cells was reduced by more than 50% if Reheis FCS was heat-inactivated at 56° for 30 minutes.

The scoring of morphologically transformed colonies requires some subjectivity in appraisal. Many colonies must be examined for transformation, especially when the transformtion frequency is not high. In addition, a battery of in vitro criteria have been tested in this laboratory to aid in scoring transformed colonies, such as the differences in staining of colonies related to the nucleus/cytoplasm ratio, peripheral cell orientation, colony growth rate and density. However, many variables, such as the health status of the dam and culture conditions, strongly influence these criteria independent of carcinogen treatment.

The direct focus assay developed by Casto and co-workers (1976) appears to be influenced by external variables and may be a reliable method for identifiying transformed colonies of cells. Chemicals including MNNG, DMBA, benzo(a)pyrene, 3-methylcholanthrene, and beta-propiolactone have induced foci. MNNG has been the most potent carcinogen tested, with transformation frequencies ranging from 200, 13.4, 5.3, to 1.5 per 10^5 survivors at MNNG concentrations of 1.5, 1, 0.5, and 0.1 μg/ml, respectively. The focus induction frequency ranges from 0.76 per 10^5 survivors in DMBA-treated cells to 200 per 10^5 survivors in MNNG-treated cells; it appears to be independent of the cell number inoculated but dependent on chemical concentration and the length of carcinogen treatment. Recent experiments have indicated that the exposure of carcinogen-treated cultures to 5% FCS about two weeks after treatment led to a 2-3 fold increase in transformation frequency as compared with cultures maintained in 10% FCS. Therefore, the expression of transformation in this system appears to be controlled by the serum-regulated cell density of the normal background cells in the cultures.

Significance to Biomedical Research and the Program of the Institute: The host mediated transplacental or in vivo-in vitro assay can produce semi-quantitative data with no spontaneous transformation frequency and can be used to test

potential carcinogenic chemicals including those which need metabolic activation or are solubilized only in organic solvent. This system can also provide a model for host-carcinogen interactions and cancer cell biology.

Date Contract Initiated: March 1, 1976

Current Annual Level: $45,042

DEPARTMENT OF ENERGY-NCI INTERAGENCY AGREEMENT (OAK RIDGE NATIONAL LABORATORY (Y01-CP-70226)

Title: Support of a Committee on Chemical Environmental Mutagens

Contractor's Project Director:

Project Officer (NCI): Dr. Virginia C. Dunkel

Objectives: This project is directed to the extrapolation of results of mutagenesis test systems to attempt to compute the effects of human populations at risk, and to proposing a basis for determining levels of exposures that minimize the risk of detrimental effects on the population. Although this effort is directed to mutagenesis, it will be of value to carcinogenesis in that the means and approaches used for establishing such guidelines may be analogously applied to the problem of risk assessment which exists in the area of carcinogenesis.

Date Contract Initiated: September 30, 1977

Current Annual Level: $21,700

UNIVERSITY OF ILLINOIS (N01-CP-23303)

Title: Temperature Sensitive Mutants in In Vitro Carcinogenesis

Contractor's Project Director: Dr. Giampiero di Mayorca

Project Officer (NCI): Dr. John Bader

Objectives: 1) Expose BHK 21 hamster cells to chemical carcinogens. 2) Analyze transformation by microscopic observation of morphological change and growth by soft agar under normally restrictive conditions such as sensitivity to temperature. 3) Manipulate clones of transformed cells for ability to grow under a variety of conditions in order to analyze the reasons for physiological changes responsible for malignancy. 4) Test transformed cells to establish the observed correlation between ability of plating in semi-solid medium and tumorigenesis in the hamster. 5) Test the feasibility of BHK cells in a quick, reliable carcinogen screening test.

Major Findings: Analysis of frame-shift mutagen-carcinogen-induced transformed BHK clones has revealed that they are all unrestricted in regard to temperature. Simultaneous dose response curves for 4NQO and NMU for mutagenesis and malignant transformation in vitro revealed almost identical kinetics with the highest frequency for both phenomena resulting at the same dose level.

Significance to Biomedical Research and the Program of the Institute: A very convincing demonstration of the mutagenic mechanism in in vitro chemical carcinogenesis is given.

Date Contract Terminated: July 1, 1978

Date Contract Initiated: June 20, 1972

Current Annual Level: $152,946

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (N01-CP-55630)

Title: The Significance of Experimental Carcinogenesis Data to Man

Contractor's Project Directors: Dr. Helmut Bartsch
Dr. Ruggero Montesano

Project Officer (NCI): Dr. Virginia C. Dunkel

Objectives: The overall objectives of this program are the development of better criteria for the extrapolation of experimental carcinogenicity data to man and the validation and improvement of rapid screening tests which may have use in selecting chemicals for in depth investigation and/or in predicting the carcinogenicity of environmental chemicals. To pursue these objectives, the research focuses on four major topics: 1) comparative metabolism of carcinogens in human and animal systems; 2) studies on DNA repair processes; 3) mutagenicity tests in vitro; 4) chemical carcinogenesis in vitro.

Major Findings: Differences in tissue-specific activation and detoxification processes of chemical carcinogens appear to be contributing factors in the production of tumors in certain organs only and may also condition the carcinogenic response in human individuals when exposed to the same level of environmental carcinogens. Inter-individual differences in the activity of carcinogen-activating enzymes were studied by measuring benzo(a)pyrene hydroxylase (AHH)

activity and microsomal-mediated mutagenicity of human liver specimens using the hepatocarcinogens, N-nitrosomorpholine, N-nitroso-N'-methylpiperazine and vinyl chloride as substrates. Vinyl chloride is converted by microsomal enzymes into an ultimate carcinogen, chloroethylene oxide, a highly electrophilic, mutagenic and carcinogenic agent. A 60-fold inter-individual variation occurred in AHH-activities and the capacity to convert vinyl chloride and the 2 nitrosamines into mutagens varied 7-17-fold. When AHH-activity in liver specimens from adult subjects was plotted against the respective microsome-mediated mutagenicity in S. typhimurium strains, a positive correlation was obtained between the rate of oxidative benzo(a)pyrene metabolism mutagenicity in the presence of vinyl chloride ($r = 0.88$; $p < 0.05$), N-nitrosomorpholine ($r = 0.84$; $p < 0.002$) and N-nitrosomorpholine ($r = 0.92$; $p < 0.002$). These results and data from animal experiments lend further support to the fact that cytochrome P450-linked mono-oxygenases in human or rat liver convert N-nitrosamines and vinyl chloride into electrophilic and mutagenic intermediates.

The microsomal AHH-activity in surgical lung specimens from patients with cancer of the respiratory tract (collaborative study) has also been investigated, drug intake and tumor histology are being collected. AHH activity in normal and tumorous lung tissues from the same patient was determined under conditions where the formation of phenolic BP metabolites were proportional to incubation time and protein concentration. Large inter-individual variations were observed in 70 patients ranging from 0.2-12 units of AHH-activity. In most cases, AHH-activity in tumorous lung tissue was lower than in normal tissue of the same patient. When AHH-activity in the tumorous tissue was plotted vs the number of cigarettes smoked per day prior to surgery, a negative correlation was obtained. These interim results suggest that differences in metabolic activation processes may condition the response of human individuals when exposed to the same level of carcinogens, 1,2-Dimethylhydrazine (1,2-DMH) induces tumors predominately of the intestinal tract in rats and other animal species. Single subcutaneous doses of 1,2-DMH to rats resulted in tumors of the colon and kidney. Within the intestinal tract, the tumors developed primarily in the colon and, less frequently, in the duodenum or rectum. In the present studies the formation of 7-methylguanine (7-meGua) and the degree of alkylation, at sites other than the 7-position in the DNA, of various tissues of rats following a single subcutaneous administration of 300 mg/kg of ^{3}H-1,2-DMH was examined. The highest level of 7-meGua was found in the DNA of the liver three hours after administration of the carcinogen. It was also detected in the DNA of the mucosa of the colon (14% of the liver value), the kidney (3.2%) and the mucosa of the ileum (1.7%). By 72 hours, the level of 7-meGua had decreased in the DNA of all the organs. At 3 hours, O^6-meGua was detected in the DNA of the liver and, to a lesser extent, in the mucosal DNA of the colon. However, at 72 hours, O^6-meGua was found only in the mucosal DNA of the colon and at the same level as that observed at 3 hours.

In order to determine if base excision repair is specific for DNA, Syrian golden hamster liver ribosomal RNA was isolated up to 96 hours after administration of [^{14}C]-dimethylnitrosamine at 25 mg/kg or 2.5 mg/kg. The chemical alkylation products, 7-methylguanine, 3-methylcytosine, O^6-methylguanosine

and 1-methyladenosine, were measured after acidic or enzymic chromatography. Between 7 and 96 hours, the relative amounts of alkylation products did not change with time, even though the absolute amounts fell by approximately 80% and 51% after the high and low doses respectively. The results, when compared to the results obtained in DNA, suggest that base specific excision repair does not exist for RNA alkylation products in this system.

Pure chemicals or complex mixtures have been tested in an in vitro system using S. typhimurium strains developed by B. N. Ames in the presence of a microsomal fraction from liver or other organs from rodents and from human specimens. Three types of assays were used: plate incorporation assays, a liquid incubation system and the plate incorporation assay adapted to test volatile compounds. Special attention has been given (1) to improving the efficiency and reproducibility of the Salmonella/microsome mutagenicity test, (2) to characterizing ultimate reactive metabolites which appear to be involved in carcinogenesis and mutagenesis, to identify in vivo possible biological reactive intermediates which cannot be isolated; and (3) testing chemicals of socio-economic importance, which have not yet been assayed for their carcinogenic activity.

The 150 compounds that have been tested for mutagenic activity in the Salmonella/microsome mutagenicity test with its adapted procedures include drugs, industrial chemicals, mycotoxins, pesticides and halo-olefins. Sixty-six compounds for which evidence of carcinogenicity exists in the literature have been found to be mutagenic. Fourteen compounds which are described as carcinogenic were not detected as mutagens. Only one false-positive result was obtained - a 'non-carcinogen' which showed mutagenic activity. Four chemicals for which there was negative evidence of carcinogenicity were not mutagenic. Of 84 compounds on which no data on carcinogenicity testing have been published, 35 were non-mutagenic and 49 mutagenic.

Tissue-mediated mutagenicity tests with S. typhimurium have also been used in studies on the mechanism of metabolic activation of polycyclic aromatic hydrocarbons, to help pin-point which particular dihydrodiol derivatives are biologically reactive precursors of vicinal diol-epoxides. The results obtained on a series of dihydrodiols derived from 7-methylbenz(a)anthracene strongly support the hypothesis that a vicinal diolepoxide, 3,4-diol-1,2-oxide, of 7-methylbenz(a)anthracene may be one biologically important metabolite of the parent hydrocarbon in vivo.

The activity of carcinogenic, chemically-reactive esters derived from N-hydroxy-2-aminofluorene, namely N-myristoyloxy-N-acetyl-2-aminofluorene, N-acetoxy-N-myristoyl-2-aminofluorene, N-myristoyloxy-N-myristoyl-2-aminofluorene and N-acetoxy-N-acetyl-2-aminofluorene were compared in short-term tests: assay for electrophilicity, mutagenicity in bacteria or V79 Chinese hamster cells and induction of unscheduled DNA repair synthesis in cultured fibroblasts.

The data showed a general qualitative correspondence between the induction of DNA repair synthesis, electrophilicity and carcinogenic activity of these esters, although quantitative differences were evident. However correlation

of these activities with mutagenicity in S. typhimurium TA-1538 and TA-98 was poor; no mutagenicity was demonstrated for the two N-myristoyloxy derivatives. These findings underline the need for carrying out multiple short-term assays in predicting the potential carcinogenic activity of chemicals.

Studies were carried out to determine whether direct or proximate contact between target cells and mediators of metabolism are necessary for the induction of mutagenesis. Two metabolic activation systems were used: cell-mediated mutagenesis (CMM), in which target cells are co-cultured with metabolically competent, but lethally irradiated feeder cells and microsome-mediated mutagenesis (MMM), in which cells are treated with chemicals in the presence of a rodent liver microsomal fraction and an NAPDH generating system.

The genetic indicator (V79 cells) were separated from the metabolic activation systems (post-mitochondrial fraction or layer of metabolizing cells) by various mechanical means. The results suggest that direct or proximate fraction or layer of metabolizing cells) by various mechanical means. The results suggest that direct or proximate contact between the target cells and membranes of the cells or microsomal particles, carrying carcinogen-metabolizing enzymes is essential in cell and microsome-mediated mutagenesis of mammalian cells.

Various criteria have been used to differentiate normal cells from cells transformed neoplastically in vitro. These criteria have been developed mainly from studies with cells of mesenchymal origin and it is questionable to what extent they are also applicable to epithelial cells. In collaboration with Dr. K. Sanford (National Cancer Institute, Bethesda, MD, USA) and Dr. B. Weinstein (Columbia University College of Physicians and Surgeons, New York, NY, USA), various epithelial cell lines, developed in the Agency laboratory, have been evaluated for their capacity to form colonies in soft agar, for their production of plasminogen activator and for morphological changes in vitro. The results have been correlated with the tumorigenic capacity of the same cell lines after injection into syngeneic hosts. The data show that growth in soft agar and some cytological changes correlated well with tumorigenicity in vivo. However, the production of plasminogen activator showed little correlation with tumorigenicity. The present data also provide evidence that plasminogen activator production is neither a necessity nor a sufficient property for growth in agar. It is possible that protease production is linked to the mechanism of metastasis formation; however, the cell cultures IAR6-1, IAR6-1-RT7 and IAR-27, which produced a high yield of metastatic lesions in the lungs when injected into rats, have a low in vitro production of plasminogen activator.

Significance to Biomedical Research and the Program of the Institute: These studies are designed to develop and evaluate various test systems for the identification of environmental carcinogens, as well as to provide criteria for a better assessment of the significance of experimental data to man.

Proposed Course: The activity will continue along the same lines as indicated in the Major Findings section.

Date Contract Initiated: September 1, 1974

Current Annual Level: $202,101

INVERESK RESEARCH INTERNATIONAL (N01-CP-75858)

Title: Validation and Utilization of Microbial Mutagenesis Systems as Prescreens for Chemical Carcinogens

Contractor's Project Director: Dr. Douglas McGregor

Project Officer (NCI): Dr. Virginia C. Dunkel
(NIEHS): Dr. Errol Zeiger

Objectives: To evaluate and validate through collaborative studies in several laboratories microbial mutagenicity and DNA repair assays for their predictive value in assessing the carcinogenic potential of chemical compounds.

Major Findings: In the initial phase of this project studies were carried out to establish the working protocol, to test the positive control compounds, sodium azide, 9-aminoacridine, 2-nitrofluorene and 2-aminoanthracene, and to prepare and evaluate the mouse, rat and hamster S-9 fractions obtained from non-induced and arochlor 1254 induced animals which would be used for metabolic activation.

The first batch of coded compounds for testing was received from the chemical repository in January 1977. Each coded compound is being tested in eight strains of bacteria, namely, Salmonella typhimurium TA-1535, 1537, 1538, 98 and 100 and Escherichia coli WP-2/uvrA$^-$ and W3110/polA$^+$ and P3478/polA$^-$, at seven dose levels both with and without metabolic activation. A base of information is systematically being collected and the decoding and comparative evaluation of the first group of 67 chemicals consisting of known carcinogens and non-carcinogens should be completed late in 1978.

Significance to Biomedical Research and the Program of the Institute: Identification of cancer-causing agents in the environment is a prerequisite for the elimination of such agents from our surroundings. The present study seeks to develop and evaluate rapid, simple and inexpensive, microbial procedures to be used as preliminary screens for chemical carcinogens.

Proposed Course: To continue testing a series of coded chemicals consisting of both carcinogens and non-carcinogens for their mutagenicity and DNA-modifying activity.

Date Contract Initiated: September 30, 1976

Current Annual Level: $89,904

JOHN L. SMITH MEMORIAL FOR CANCER RESEARCH PFIZER, INC. (N01-CP-55703)

Title: Development of Detailed Methods and Protocols for Carcinogenesis Screening Using Cell Culture Assays-Task I - Fischer Rat Embryo Cells Infected with Rauscher Leukemia Virus - Task III - Fischer Rat Embryo Cells

Contractor's Project Director: Dr. John S. Wolff, III

Project Officer (NCI): Dr. Virginia C. Dunkel

Objectives: Develop methodology, using normal or Rauscher leukemia virus (RLV) infected rat embryo cells, suitable for in vitro assessment of the carcinogenic potential of chemicals. Validate tests by assessing susceptibility to a series of coded chemicals consisting of both carcinogens and non-carcinogens.

Major Findings: Culture strains of Fischer rat embryo cells infected with Rauscher leukemia virus are susceptible to transformation by carcinogenic chemicals, but are unaffected by noncarcinogens. An assay has been developed which is sufficiently sensitive so that some compounds of uncertain in vivo carcinogenicity (e.g. 1,2-epoxybutane, diphenylnitrosamine) have been detected as positive. Certain chemicals (e.g. urethane) revealed in other types of assays only after metabolic activation, if at all, are clearly active in this test. Sensitivity to induction of foci by 3-methylcholanthrene is enhanced 5-10 fold by addition of the tumor promotor phorbol acetate. Preliminary data suggest that similar enhancement of sensitivity may be gained by inclusion of a liver S-9 metabolizing system. To ensure that all compounds are tested at concentrations that comprise a range of biologically active doses, toxicity is determined in a high through-put, Multiwell plate assay. Transformation tests are performed with concentrations that are LD_{20}, LD_2 and $LD_{0.2}$. Using this approach, diethylnitrosamine, a weak in vivo carcinogen previously found negative at a standard dose which was found to be less than $LD_{0.5}$, induced focus formation at higher LD levels.

Transformation has been validated by observing ability of focus-forming cells to grow in soft agar. A modified test is now in use in which transformed, but not normal, cells are observed to survive when grown over a solid agar base. This technique offers quick (6 day) validation and is now being assessed for utility as a rapid (10 day) assay for chemical carcinogenicity. Several known carcinogens have been positive in the assay and this novel method is now being intensively studied for broader application.

Assay of coded compounds in $2FR_450$ cells has begun, with toxicity data completed for 45 chemicals. Eleven compounds are on test and 5 have been terminated after identification and validation as carcinogens.

Several strains of uninfected rat embryo cells have proven resistant to carcinogen-induced transformation. Recent data suggest that addition of phorbol accetate to 3-MC treatment resulted in morphological alteration of target cells. Extension of these studied is in progress.

Significance to Biomedical Research and the Program of the Institute: Each year, many new chemicals are synthesized for use in consumer products or as intermediates in other processes. Some unknown number of these is likely to be carcinogenic. Present bioassay protocols are too expensive and time-consuming to permit carcinogenicity assay of each of these new compounds. It is expected that a prescreen, combining in vitro carcinogenicity assay with other procedures, will identify compound candidates for bioassay. The system utilizing RLV-infected rat embryo cells has proved to be sensitive to transformation by many carcinogens. Development of in vitro carcinogenesis protocols using this model should provide one means of bioassay prescreen.

Proposed Course: 1) Continue assay validation with RLV-infected rat embryo cells with 90 coded compounds. 2) Continue investigation of growth characterisatics of RLV-infected cells, with the objective of developing a more rapid, more sensitive assay. 3) Determine utility of non-virus-infected rat cells for carcinogen assay.

Date Contract Initiated: June 23, 1975

Current Annual Level: $230,562

LEO GOODWIN INSTITUTE FOR CANCER RESEARCH (N01-CP-65804)

Title: Production of an In-Bred Syrian Hamster Colony

Project Director: Ms. Miriam R. Sacksteder

Project Officer (NCI): Mr. Clarence Reeder

Objective: To maintain, breed and monitor a colony of 1500 Graffi strain Syrian hamsters (Ca 400 ♂ and 1100 ♀). To distribute as directed by the Project Officer timed pregnant females, excess weanlings as well as retired breeders.

Major Findings: During the period 4/1/77 - 3/1/78, 323 timed pregnant females, 269 weanlings and 112 retired breeders were distributed to investigators for chemical carcinogenesis studies. There have been no enteric pathogens, infections or other unusual health problems in the colony. Oxyuris vermicularis (pin worms) has been detected in a small number of individuals and a medicated diet will be introduced to eliminate this parasite.

Significance to Biomedical Research and the Program of the Institute: The transformation of cells in vitro provides a means by which a large number of chemicals could be tested for carcinogenic potential. Studies with Syrian hamster embryo cells have shown that there is a good correlation between in vitro transformation in these cells and the in vivo carcinogenic activities of the compounds tested. The availability of a supply of hamsters of excellent quality will assure that continuing and uninterrupted studies can be carried out with assay systems which use these cells.

Proposed Course: To continue to breed, monitor and distribute Syrian hamsters to investigators in need of high quality of animals.

Date Contract Initiated: September 1, 1976

Current Annual Level: $91,311

LITTON BIONETICS, INCORPORATED (N01-CP-65856)

Title: Validation and Utilization of Microbial Mutagenesis Systems as Prescreens for Chemical Carcinogens

Contractor's Project Director: Dr. David Brusick

Project Officer (NCI): Dr. Virginia C. Dunkel
(NIEHS): Dr. Errol Zeiger

Objectives: To evaluate and validate through collaborative studies in several laboratories microbial mutagenicity and DNA repair assays for their predictive value in assessing the carcinogenic potential of chemical compounds.

Major Findings: In the initial phase of this project studies were carried out to establish the working protocol, to test the positive control compounds, sodium azide, 9-aminoacridine, 2-nitrofluorene and 2-aminoanthracene, and to prepare and evaluate the mouse, rat and hamster S-9 fractions obtained from non-induced and arochlor 1254 induced animals which would be used for metabolic activation.

The first batch of coded compounds for testing was received from the chemical repository on January 1977. Each coded compound is being tested in eight strains of bacteria, namely, Salmonella typhimurium TA-1535, 1537, 1538, 98 and 100 and Escherichia coli WP-2/uvrA$^-$ and W3110/polA$^+$ and p3478/polA$^-$, at seven dose levels both with and without metabolic activation. A base of information is systematically being collected and the decoding and comparative evaluation of the first group of 67 chemicals consisting of known carcinogens and non-carcinogens should be completed late in 1978.

Significance to Biomedical Research and the Program of the Institute: Identification of cancer-causing agents in the environment is a prerequisite for the elimination of such agents from our surroundings. The present study seeks to develop and evaluate rapid, simple and inexpensive, microbial procedures to be used as preliminary screens for chemicals carcinogens.

Proposed Course: To continue testing a series of coded chemicals consisting of both carcinogens and non-carcinogens for their mutagenicity and DNA-modifying activity.

Date Contract Initiated: September 30, 1976

Current Annual Level: $172,240

LITTON BIONETICS, INCORPORATED (NO1-CP-65853)

Title: Development and Validation of an In Vitro Mammalian Cell Mutagenesis System for Carcinogenesis Screening

Contractor's Project Director: Dr. Dale W. Matheson

Project Officers (NCI): Dr. Virginia C. Dunkel
(FDA): Mr. Kenneth Palmer

Objectives: To evaluate and determine the usefulness and reliability of an in vitro mutagenesis assay system using L5178Y mouse lymphoma cells (TK^{+}/- locus) as a prescreen for potential chemical carcinogens.

Major Findings: The initial effort on this project has been directed to the development of the L5178Y mouse lymphoma cell mutagenicity assay. The studies to date have proceeded in four phases. In the first phase and prior to testing of chemicals, studies were carried out to demonstrate that the cells would grow at the reported 4 to 5 doubling rate in 24 hours and could be cloned in soft agar successively at a rate of greater than 75%. In the second phase, six reference chemicals, namely ethyl methanesulfonate (EMS), hycanthone, methyl methanesulfonate (MMS), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), para-rosaniline, and phenanthrene) were tested in the absence of metabolic activation in order to define a suitable protocol. It was determined at this time that changes in assay conditions were required and these included the use of a two-day expression period instead of a three-day expression time and the use of trifluorothymidine (TFT) instead of bromodeoxyuridine (BUdR) as the selective agent for mutant colonies.

Work in the third phase entailed 1) development of metabolic activation methodology using S-9 preparations from the livers of uniduced and Arochlor 1254-induced Fischer rats, and 2) evaluation of ten additional compounds without metabolic activation and with S-9 preparations from both uninduced and induced rats. The chemicals tested in this phase were 2-acetylaminofluorene, benzo(a) pyrene, 7,12-dimethylbenz(a)anthracene, diethylnitrosamine, dimethylnitrosamine, diphenylnitrosamine, 3-methylcholanthrene, 2-methyl-4-dimethylaminoazobenzene, 2-naphthylamine and pyrene.

Phase IV studies are now underway in which all 16 of the above reference chemicals are being retested both with and without metabolic activation.

Significance to Biomedical Research and the Program of the Institute: Since the number of potentially hazardous chemicals that may significantly affect human population groups or the general population far exceeds the capacity of existing long-term animal and carcinogenesis test systems, there is a need for rapid short-term tests such as the mouse lymphoma mutagenesis assay system that may be used in a battery for the initial evaluation of chemicals for possible carcinogenic potential.

Proposed Course: Sufficient confidence with the system has been gained so that in the immediate future approximately 100 chemicals consisting of both carcinogens and noncarcinogenic analogues will be tested blind to determine and validate the response of the system.

Date Contract Initiated: September 30, 1976

Current Annual Level: $205,717

MICROBIOLOGICAL ASSOCIATES (N01-CP-55670)

Title: Development of Detailed Methods and Protocols for Carcinogenesis Screening Using Cell Culture Assays - Task I Fischer Rat Embryo Cells Infected with Rauscher Leukemia Virus

Contractor's Project Director: Dr. Nirmal K. Mishra

Project Officer (NCI): Dr. Virginia C. Dunkel

Objectives: To develop and determine the usefulness and reliability of an in vitro cell transformation system using Fischer rat embryo cells infected with Rauscher leukemia virus for screening chemicals for carcinogenic potential.

Major Findings: A multicomponent test system using Fischer rat embryo (FRE) cells preinfected with Rauscher leukemia virus (RLV) is being standardized as an assay system for the detection of chemical carcinogens. The endpoints measured are gene mutation (ouabain resistence) and cellular transformation. Eight known carcinogens and five structurally related non-carcinogenic analogues were tested in this system for standardization purposes. With the exception of one carcinogen, namely, N-2-fluorenylacetamide, all carcinogenic chemicals induced transformation. Furthermore, all transforming agents produced stable ouabain resistant cellular subpopulations.

Using morphological transformation as the single endpoint, chemicals have been and are being tested in blind studies. Assays on approximately twenty-five different coded chemicals have been completed.

Since there is the need for exogenous metabolic activation in this system, attempts are presently being made to standardize liver homogenates in terms of specific components of the total enzymatic activity such as aryl hydrocarbon hydroxylase. Using N-2 fluorenylacetamide and dimethylnitrosamine as the model procarcinogens, it has been found that clarified liver homogenates (S-9 fraction) can be used as a source of activating enzymes. However, the input doses of S-9 homogenate which are effective when used with DMN are not useful for N-2-fluorenylacetamide.

Some basic inquiries regarding the role of leukemia virus in the promotion of transformation and mutagenic activity in the test have been made. Although RLV infection distinctly suppressed cellular post replication repair after chemical assault, and also enhanced the rate of transformation in FRE cells by chemical carcinogens, chemically induced gene mutation rates were not modified by virus infection.

Significance to Biomedical Research and the Program of the Institute: Each year, a significant number of new chemicals are synthesized and many of these are used in ways that may significantly affect specific population groups or the general population. Since the numbers of chemicals which require listing

far exceeds the capacity of existing long-term animal carcinogenesis test systems the need exists to define the reliability of rapid sensitive and reproducible short-term assays. This transformation system which uses Fischer rat embryo cells infected with Rauscher leukemia virus is sensitive to many chemical carcinogens and could thus, in conjunction with other procedures be used as a prescreen for determining the carcinogenic potential of chemical compounds.

Proposed Course: 1) To complete assay of coded samples supplied by NCI; 2) To improve the sensitivity of the assay by determining the conditions required for metabolism of various procarcinogens; and 3) To improve the reliability of the assay system with known chemicals.

Date Contract Initiated: June 27, 1975

Current Annual Level: $208,058

NCI - Frederick Cancer Research Center (Litton Bionetics, Incorporated)
Project #10 (NO1-CO-25423)

Title: In Vitro Carcinogenesis

Contractor's Project Director: Dr. Roman J. Pienta

Project Officer (NCI): Dr. Virginia C. Dunkel

Objectives: To develop standardized in vitro bioassay methods for detecting potential carcinogens and studying mechanisms of carcinogenesis. To define standard experimental conditions and endpoints so that the system is reproducible rapid and valid and can be readily used in other laboratories.

Major Findings: In Vitro Carcinogenesis Bioassay: A hamster embryo cell carcinogenesis bioassay previously standardized by using pretested aliquot samples of primary cultures cryopreserved in liquid nitrogen as the source of target and X-irradiated feeder cells, was further evaluated for its reliability. Thus far, 106 reference chemicals have been tested. Transformation was observed with most carcinogens (92%) and no false positives were observed when cells were treated with noncarcinogens. Spontaneous transformation was never observed in control cultures treated with solvent or culture medium alone. When liver homogenate enzymes were added to eight carcinogens apparently requiring further metabolic activation, seven of these induced transformation. It has also been possible to metabolically activate two of the compounds to transform cells by incorporating hamster hepatocytes into the bioassay procedure. These modifications are being further standardized to enhance the responsiveness of the bioassay and thereby eliminate apparently false negative responses.

The bioassay was also used to assess the carcinogenic potential of several drugs, food additives and endogenous materials produced in the gut. Bioassay of the first ten compounds revealed three carcinogens, that were identified by the NCI after results were submitted. Several other compounds of special

interest namely, saccharin, dl-amygdalin, and methyapyrilene, were bioassayed and were negative in the standard test. However, when a reaction mixture resulting from treatment of methapyrilene with nitrite was tested in the prescence of a hamster liver homogenate transformation of the hamster cells did occur.

Preliminary experiments have also shown that hamster cells could be transformed in vitro by metabolites extracted from glucuronidase-treated urine from rats treated with N-2-fluorenylacetamide (AAF). This model system may have use in monitoring selected human population groups for exposure to carcinogens. Metabolism of N-acetylaminofluorene as a Marker for Identifying and Assessing Exogenous Metabolic Activation Systems: The metabolism of AAF to the proximate carcinogen N-hydroxy-AAF was used as a biological marker to assess the efficacy of candidate feeder cells or microsomal enzyme preparations in continued effort to standardize methods for enhancing the sensitivity of the bioassay. It has been observed that the hydroxylation of AAF occurred at a rate 50 times greater in cultured hamster hepatocytes than in either hamster embryo cells or human embryo cells. Hamster microsomes catalyzed the formation of N-hydroxy-AAF at the rate of 296 nmoles per mg protein per hour. The deacetylation of AAF to AF by hamster hepatocytes was 500 times greater than that observed with hamster embryo cells and 70 times greater than that observed with human embryo cells. Cells treated in suspension metabolized AAF to N-hydroxy AAF at the same rate as cells treated in monolayer cultures. The low rate of N-hydroxy-AAF formation observed with both hamster and human embryo cells agrees with the inability to malignantly transform these cells with AAF in the absence of metabolic activation provided by liver homogenate (S-9) enzymes.

Identification of Reliable Sources of Target Cells: Cell cultures prepared either from individual or pooled embryos vary in their susceptibility to transformation by carcinogens. This problem has been minimized by the use of cryopreserved cultures that were previously selected for their susceptibility to transformation. However, to identify sources of more reliable cells or determine why some batches of cells are refractory to transformation a methodical evaluation of hamster embryo cells from several commercial suppliers, has been initiated. So far, it has been observed that cells from only 25% of the embryos responded to transforming doses of 3-MC. In more recent studies eight pools of cells from two suppliers were examined for transformation by benzo(a)pyrene and for benzpyrene hydroxylase (BPH) activity. Fifty percent of these pools were transformed by the carcinogen. When these cell pools were induced with benz(a)anthracene, those which transformed were found to produce about 100 pmoles of 3hydroxy BP/mg protein/ 30 minutes more than those which did not transform. This parameter BPH activity, appears to correlate with the transformability of the cells by this polycyclic aromatic hydrocarbon. Correlation with other classes of carcinogens is under investigation.

The Binding of Carcinogens to Macromolecules of Cells in Culture: Various factors affecting the binding of DMBA, DB(a,c)A, and DB(a,h) A to human embryo cells and either transformable or nontransformable cultured hamster embryo

cells were studied. As the generation at which cells are used increased, a dramatic decrease in the binding of DMBA to hamster embryo cell DNA, RNA, and protein (>100-fold) occurred and a concomitant decrease in transformation frequency was observed. The best correlation with carcinogenicity and transforming activity was observed with the binding of DMBA to hamster embryo cell DNA and RNA. The binding of DMBA, DB(a,h)A, and DB(a,c)A to DNA and RNA of human embryo cells was low at all generations while binding of these carcinogens to cellular protein generally occurred to a much greater extent. The effect of serum concentration and cell confluence on the extent of binding was also studied. With hamster embryo cells, the amount of serum in the medium did not influence binding to DNA, RNA or protein. The binding of DMBA to cells at 70% confluence was 10-fold greater than to cells at 100% confluence. With human embryo cells, DB(a,h)A binding to RNA and protein decreased slightly when the serum concentration was increased from 5% to 10%. Increased confluence of the cells resulted in a slight increase in DMBA and DB(a,h)A binding to cellular macromolecules. This contrasts with hamster embryo cells in which binding decreased to basal levels when the cells were treated at 100% confluence.

Effects of a Tumor Promoting Agent on Transformation: To determine whether a promoting agent could be used for enhancing the response of the bioassay, the effect of 12-O-tetradecanoyl-phorbol-13-acetate (TPA) on transformation in mass cultures was studied. It was observed that morphological transformation of hamster embryo cells could be either inhibited or enhanced depending upon the dose of carcinogen used as well as the time between treatment with carcinogen and promoter. Time of treatment with promoter also affected other characteristics of the transformed hamster cells such as growth in soft agar and tumorigenicity in hamster. When added simultaneously with carcinogen, TPA also enhanced cloning efficiency and the incorporation of thymidine-^{3}H. Cells maintained continuously on TPA exhibited a prolonged life span but became senescent when treatment with TPA was discontinued.

Studies with Human Cells: Early passage cultures of human foreskin cells were synchronized and sequentially treated with 4-aminobiphenyl, 4-nitroquinoline-1-oxide, and beryllium sulphate. After three cycles of treatment the cells lost post-confluence inhibition, exhibited an accelerated growth rate, and could grow as three dimensional colonies in soft agar. Subcultured samples of transformed and untreated cells were cryopreserved and are being evaluated for malignancy in athymic nude mice and for other in vitro criteria for transformation.

Significance of Biomedical Research and the Program of the Institute: A major objective of the National Cancer Program is the identification of potentially carcinogenic chemicals posing a hazard to man so that these agents can be removed from the environment. This becomes more important as more chemicals are introduced each year, thereby increasing the number of potentially carcinogenic chemicals which should be identified. The In Vitro Carcinogenesis Project at FCRC emphasizes the development of reliable short-term carcinogenesis bioassays that could be used for prescreening chemicals and selecting those requiring further testing in animals. The hamster embryo cell in vitro

carcinogenesis bioassay shows promise of being such a system because of the high degree of response concurring with conventional in vivo bioassay. The system can also be used to study the mechanism of action of carcinogens so that steps might be devised to inhibit or interrupt the process and thus reduce the incidence or modify the course of cancer.

Proposed Course: Efforts will continue to identify optimal experimental conditions for obtaining maximum susceptibility to transformation of cells by carcinogens. Transformation by a broader range of classes of chemical carcinogens will be investigated to establish the usefulness and limitations of the system. Continued emphasis will be placed on enhancing the sensitivity of the system to eliminate the false negative responses seen with several carcinogens by incorporating exogenous metabolic activation systems such as microsome enzymes or hepatocytes, or by modifying the experimental design and cultural conditions of the system. This should allow the system to detect weak carcinogens and help to assign potencies of chemicals with unknown carcinogenic potential. The metabolism of selected carcinogens by microsome enzymes form several mammalian species, including man, will be continued in order to gain insight into the process of carcinogenesis and to make the bioassay more relevant to identifying carcinogens hazardous to man.

Date Contract Initiated: June 26, 1972

Current Annual Level: $450,000

NEW YORK MEDICAL COLLEGE (NO1-CP-65855)

Title: Validation and Utilization of Microbial Mutagenesis Systems as Prescreens for Chemical Carcinogens

Contractor's Project Directors: Dr. Herbert S. Rosenkranz
Dr. Elena McCoy

Project Officer (NCI): Dr. Virginia C. Dunkel
(NIEHS): Dr. Errol Zeiger

Objectives: To evaluate and validate through collaborative studies in several laboratories microbial mutagenicity and DNA repair assays for their predictive value in assessing the carcinogenic potential of chemical compounds.

Major Findings: In the initial phase of this project studies were carried out to establish the working protocol to test the positive control compounds, sodium azide, 9-aminoacridine, 2-nitrofluorene and 2-aminoanthracene, and to prepare and evaluate the mouse, rat and hamster S-9 fractions obtained from non-induced and arochlor 1254 induced animals which would be used for metabolic activation.

The first batch of coded compounds for testing was received from the chemical repository on January 1977. Each coded compound is being tested in eight strains of bacteria, namely, Salmonella typhimurium TA-1535, 1537, 1538, 98 and 100 and Escherichia coli WP-2/uvrA$^-$ and W3110/polA$^+$ and p3478/polA$^-$ at

seven dose levels both with and without metabolic activation. A base of information is systematically being collected and the decoding and comparative evaluation of the first group of 67 chemicals consisting of known carcinogens and non-carcinogens should be completed late in 1978.

Significance to Biomedical Research and the Program of the Institute: Indentification of cancer-causing agents in the environment is a prerequisite for the elimination of such agents from our surroundings. The present study seeks to develop and evaluate rapid, simple and inexpensive, microbial procedures to be used as preliminary screens for chemical carcinogens.

Proposed Course: To continue testing a series of coded chemicals consisting of both carcinogens and non-carcinogens for their mutagenicity and DNA modifying activity.

Date Contract Initiated: September 30, 1976

Current Annual Level: $89,904

UNIVERSITY OF NORTH CAROLINA (NO1-CP-55707)

Title: Development of Detailed Methods and Protocols for Carcinogenesis Screening Using Cell Culture Assays - Task V - Epithelial Cells

Contractor's Project Director: Dr. Joe W. Grisham

Project Officer (NCI): Dr. Virginia C. Dunkel

Objectives: The objectives of this contract are to develop methods and procedures for detecting chemically-induced transformation in cultured liver epithelial cells and to use this system to screen reference chemicals (both carcinogens and their noncarcinogen analogues) supplied by the National Cancer Institute.

Major Findings: Work on this project has demonstrated, that continuous lines of liver epithelial cells can be adapted to form the basis of a rapid in vitro system to screen chemicals for carcinogenic potential. The key finding, which allows this conclusion, is the observation that neoplastic transformation in cultured liver epithelial cells in vitro is a step-wise process, with multiple definable stages beginning when cells are exposed to carcinogens and culminating when the treated cells develop the capacity to produce tumors on back transplantation into isogeneic hosts. The transformation sequence in vitro, as defined in this study, appears analogous to the multistage process of hepatic carcinogenesis in vivo. Step-wise, programmed stages may characterize epithelial carcinogenesis generally, and further examination of the in vitro transformation sequence may provide new insights into the cellular biology of epithelial neoplasia.

Already, understanding of the transformation sequence in vitro may provide the basis for a more rapid in vitro screening system employing cultured liver epithelial cells. Tumorigenicity is a late event in the transformation

sequence in epithelial cells requiring for its development the steady proliferation of initiated cells for several generations in vitro (up to 6 months). Other relatively late stages include the ability to grow in soft agar, alterations in colony morphology, and aneuploidy. Although ability to grow in soft agar and altered colony morphology recently have been proposed as useful markers of transformation in vitro, these events also occur late in the transformation sequence, requiring almost as long to develop as does tumorigenicity. Therefore, these parameters could not form the endpoint for a rapid screening system using cultured liver epithelial cells. Several permanent changes in cellular behavior occur soon after carcinogen exposure in vitro, such as augmented growth, increased saturation density, and increased colony forming ability. These changes appear to be indelibly linked to ultimate tumorigenicity. These early alterations in cell behavior may provide useful endpoints for a carcinogen screening system that retains the precision and accuracy of assay systems using tumorigenicity as the endpoint, but with a significant gain in speed when compared to the later.

Another important finding of this project supporting the use of cultured liver epithelial cells for screening procarcinogenic chemicals, is our observation that cells from several hepatic epithelial lines metabolize benzo (a)pyrene more effiencntly than previously had been thought. In fact, when corrections for differences in cellular mass are made, some cultured liver epithelial cells metabolize benzo(a)pyrene at a rate equivalent to freshly isolated hepatocytes. Based on preliminary observations they may also be able to metabolize representative of other chemical classes.

A finding of this study, potentially important for a proper understanding of regulation of proliferation of normal and transformed liver epithelial cells, is the observation of the requirement for cell contact, apparently through the mediation of gap junctions, for proliferation to occur. Previous studies have demonstrated that adjacent diploid cells in a sheet of cultured liver epithelium are electronically coupled, and that electronic coupling is lost in tumorigenic cells. Studies indicate that intimate cell contact between two or more diploid liver epithelial cells is required before they can proliferate. Loss of electronic coupling and improved colony forming efficiency may be associated, both perhaps following obliteration or alteration in gap junctions.

Proposed Course: Cultured hepatic cells (primaries and continuous lines) will be exposed to selected carcinogenic chemicals and appropriate non-carcinogenic analogues under defined conditions. Using as endpoints the occurrence of DNA damage and repair in primary hepatocytes, evaluated by a new technique, and the occurrence of selected early stages of the transformation sequence in continuous liver epithelial lines, such as elevated saturation density and increased CFE, we will develop rapid screening procedures. Screening systems will be tested by thoroughly evaluating the effects of five or six prototypical carcinogens, representing both pro and ultimate carcinogens and their non-carcinogenic analogues. Doubleblind experimental formats will be employed. After gaining familiarity with selected systems a series of unknown chemicals, supplied by the National Cancer Institute, will be assayed.

Significance to Biomedical Research and the Program of the Institute: The major goal of this project is to develop rapid and reproducible screening assays for chemical carcinogens in epithelial cultures. Assessment of end points such as DNA damage and repair in primary cultures of hepatocytes and evaluated saturation density and increased CFE in continuous rat liver epithelial cultures may provide suitable screening systems. Thus, it may be possible to use these systems for evaluating the potential carcinogenicity of chemicals and establishing priorities for bioassay.

Date Contract Initiated: June 30, 1976

Current Annual Level: $101,799

OHIO STATE UNIVERSITY (NO1-CP-43276)

Title: In Vitro Study of the Nature of the Interaction Between Chemical and Viral Carcinogens

Contractor's Project Director: Dr. George Milo

Project Officer (NCI): Dr. Joseph A. DiPaolo

Objectives: To determine whether known chemical carcinogens will induce morphologic and neoplastic transformation of selected human cells in vitro; and to determine whether hormones that enhance cell proliferation will facilitate the transformation by chemical carcinogens. To examine and evaluate different indices of in vitro transformation that can be used to correlate in vitro with in vivo activities. To develop presumptive indices to identify which cell populations transformed by chemical carcinogen prior to their selection by passage in soft agar.

Major Findings: Cell cultures prepared from foreskin tissue were treated at low passage with a variety of carcinogens, 4-nitroquinoline-1-oxide (4NQO), N-methyl-N'-nitro-N-nitroso guanidine (MNNG), N-acetoxy-N-2-fluorenylacetamide (N-Ac-AAF), aflatoxin B_1 (AfB_1), propane sultone (PS), 3-methylcholanthrene (MCA), 7,12-dimethylbenzanthracene (7-12DMBA), benzo(a)pyrene (B(a)P, 1-napthylamine (1NA), and 2-napthylamine (2NA).

Cells exposed to one, repeated sequential, or continuously to 4-NQO, MNNG, N-Ac-AAF, AfB_1, P. S., B(a)P, and 1-NA resulted in altered colony morphology, extension of lifespan, growth in semi-solid media, altered lectin agglutination profiles, altered susceptibility to KB cell lysates and produced tumors when injected into suitable hosts. In addition, AfB_1, B(a)P, P.S., 7,12-DMBA, 1-NA and 2-NA stimulated the proliferation rate as evidenced by an increase in the rate of incorporation of ^{3}H-thymidine into cellular DNA. The carcinogens AfB_1, B(a)P, P.S., N-Ac-AAF, 1-NA, 4-NQO, MNNG, also induced unscheduled DNA synthesis. No detectable cellular or molecular responses elicited by 3-MCA while 7,12-DMBA stimulated scheduled DNA synthesis but did not induce unscheduled DNA synthesis. Treatment of human foreskin cell populations with different

carcinogens and 17$_\beta$-estradiol, phorbclymristate-acetate, anthranilin or diethylstilbesterol resulted in foci of altered cells from which "transformed" cell lines are derived. When cells at a low population doubling (PDL 1-5) and also in the S phase of the cell cycle were treated with carcinogen and hormone the cultures exhibited altered culture characteristics by PDL 20. At the present, 37 different morphologically transformed cell lines have been recovered. These cell lines grow at 41^oC, grow in the absence of CO_2, form colonies in soft agar, grow in 1% FBS supplemented growth medium and reach confluence in 50% - 67% less time when serially subpassage 1:4 than control cells. Injection of transformed cells into suitable hosts yields tumors in 4-6 weeks after injection.

Activation mechanisms were induced by B(a)P and DMBA in low passage human lung and foreskin cells but not by 3-MCA. Hydroxylated metabolites were formed and identified as being present in the cell by TLC.

The research on the virus-carcinogen system revealed that the carcinogens identified above which induced transformation also augmented SV-40 virus and feline sarcoma virus-induced transformation. All foci formed in this carcinogen system were identified by an agglutination assay as virus-directed foci.

Significance to Biomedical Research and the Program of the Institute: An end result of screening chemicals for carcinogenic potential is to protect the human population. In general most assays used for such assessments use rodents or rodent cells and from the results of these tests there must be an extrapolation of the risk to man. The work conducted on this project shows that human cells can be transformed by chemicals and provides a means to aid in human risk assessment.

Date Contract Terminated: September 1, 1978

Date Contract Initiated: March 4, 1974

Current Annual Level: $139,902

UNIVERSITY OF SOUTHERN CALIFORNIA (NO1-CP-65831)

Title: Carcinogenesis in Vitro: Initiation and Promotion

Contractor's Project Director: Dr. Charles Heidelberger

Project Officer (NCI): Dr. Virginia C. Dunkel

Objectives: To develop the C3H 10T 1/2 line of mouse embryo fibroblasts to the point where it would be feasible to evaluate its potential as a pre-screen for environmental carcinogens. The basic goals of the project are: 1) to develop a liver cell or liver microsomal activating system; 2) to improve the

quantitation of scoring for oncogenic transformation by the uptake of tritiated thymidine following confluence, the use of soft agarose, plasminogen activator, and microdetection of common embryonic antigens; 3) to develop an initiation-promotion system as a potential pre-screen for carcinogens; and 4) to develop methods for the assay of sny-carcinogenesis as a potential pre-screen for combinations of weak carcinogens.

Major Findings: C3H 10T 1/2 cells are transformable by 3-methylcholanthrene (MCA), benzo(a)pyrene (BP) and 7,12-dimethylbenz(a)anthracene (DMBA) at 0.25 μg/ml. When the cells were treated with 0.1 μg/ml of the above carcinogens, there was no or very little transformation; this was a subeffective concentration. The tumor promoting phorbol ester, 12-O-tetradecanoyl-phorbol-13-acetate (TPA), was found to be nontoxic and nontransforming to these cells at a concentration of 0.1 μg/ml. However, when the cells received 0.1 μg/ml of TPA 5 days after 0.1 μg/ml of the above hydrocarbons the frequency of transformation was highly significant. By contrast, in cultures that received TPA immediately after the treatment with three hydrocarbons in transforming (0.25 μg/ml) concentrations the frequency of transformation was diminished. It was found that the above amount of TPA inhibited cell proliferation for 24 hours, thus, it appeared that the transformation of those cells that was initiated by hydrocarbons could be promoted by TPA treatment provided that the initiated cells were allowed to undergo division to allow "fixation" of the transformed state. In addition to TPA, other phorbol esters were examined for their promoting activity. PDD and 4-α-PDD were found to be weak promoters and phorbol was inactive. When TPA treatment preceded MCA treatment no enhancement of transformation was observed.

In addition to chemical carcinogens, two physical carcinogens, ultraviolet light (UV) and X-irradiation were found to initiate the cells in the process of oncogenic transformation. The C3H 10T 1/2 cells were irradiated once with 10, 25, 100, 150 and 200 ergs/mm^2 from a UV light. No transformation was observed at any of the above doses after six weeks of culture, and no cytotoxicity at the lowest two doses. However, when the irradiated cells were cultured in a medium containing 0.1 μg/ml of TPA starting from 0 to 120 hours after the irradiation, a high frequency of transformation was produced in all cases. When the cells were initiated with subeffective concentrations of MCA (0.1 μg/ml), followed by UV irradiation at different intervals, no transformation occurred. Moreover, different doses of UV irradiation followed by 0.1 μg/ml of MCA or multiple applications of UV irradiation of different doses at different intervals did not yield transformation. Thus, it is evident that UV light acts as a pure initiator and promoting agent is required for the completion of the transformation process. When the cells were treated first with TPA, and then with UV light, there was little or no transformation. It was also found that the stimulus to cell proliferation after initiation was not sufficient to promote the transformation, although it was necessary. Therefore, TPA exerts some effect in addition to the stimulation of cell division.

The molecular events taking place after initiation and promotion were considered. Initially, DNA synthesis was measured both by autoradiography and

by the incorporation of tritiated thymidine into DNA of the cells treated with MCA, UV light, or different active and inactive promoters, i.e., different phorbol esters. It was found that active promoters produced an inhibitory action on thymidine incorporation for about 12 hours after application, and then there was an apparent compensatory increase, which was not a real stimulation. The inactive promoters had no such effect.

It has also been found with C3H 10T 1/2 cells that nontransforming doses of the same or different polycyclic hydrocarbons can be added with proper timing, and produce significantly increased transformation. This syn-carcinogenesis was most marked when the cells were treated with nontransforming concentration of MCA (0.1 μg/ml) and 1-3 days later with the same nontransforming concentration of MCA BP or DMBA. When the cells were treated first with a nontransforming concentration of BP or DMBA and then with the same concentration of the other compound at different intervals, the effect was less marked.

It has been shown that TPA can promote x-ray induced transformation. The promotion was particularly marked when a minimal transforming x-ray dose (50 or 100 rads) was followed by TPA treatment either immediately or 96 hours after irradiation.

A major finding has been that saccharin acts as a promoter of transformation in C3H 10T 1/2 cells. Highly purified saccharin at a very high concentration (2 mg/ml) did not transform the cells. However, when the initiated cells (produced by treatment with 0.1 μg/ml of MCA) were treated with 100 μg/ml of saccharin repeatedly during each medium change, the cells were transformed. On the other hand, the cells initiated with UV could not be transformed with repeated saccharin treatment. On a weight basis, saccharin is an active promoter, but only 1/1000 as active as TPA.

The following compounds are currently being tested for initiating, promoting, and complete transforming activity in the C3H 10T 1/2 cell system: cholesterol α-epoxide, anthralin, Tween 60, concanavalin A, and epidermal growth factor. In addition the antipromoting activites of several glucocorticoid steriods, and the protesase inhibitor, antipain are being determined.

TPA also inhibits 2-deoxyglucose uptake of nontransformed C3H 10T 1/2 cells both at log and confluent stage of growth, while it increased the uptake in a transformed clone both at log and confluent stages of growth. Other malignant cells tested showed the same response. The effects of TPA and other promoters on RNA and protein synthesis of C3H 10T 1/2 cells is now under investigation. A preliminary experiment showed that TPA increased RNA synthesis but decreased protein synthesis.

The ability of cells to grow in semi-solid medium is currently being studied using agarose as a suspending agent; agar was highly cytotoxic to chemically transformed cells. More than 20 chemically transformed fibroblast cells have been examined. All those that are oncogenic upon inoculation into suitable

host animals are capable of growing in 0.3% agarose. Some cell lines have been isolated which grow in agarose but are not oncogenic. The ability to grow in agarose frequently appears to exhibit a dependence upon the passage number of the cells. Agarose plating efficiencies are low shortly after chemical transformation and increase as the cells are passaged. The C3H 10T 1/2 parent line shows no capacity to grow in agarose regardless of passage number.

In a series of experiments it was found that C3H 10T 1/2 cells with added liver microsomes could metabolize aflatoxin B_1 and dimethylnitrosamine (DMN), as evidenced by cytotoxicity. The 9000 x g supernatant of homogenized rat liver was used as the enzyme preparation. This with added co-factors (NADP, NADPH, G-6P, and NADH) was mixed with PBS(HEPES) containing the carcinogens, which was then added quickly to the culture. The treatment time was 7 hours. Several preliminary transformation experiments with these compounds, which are not metabolized by C3H 10T 1/2 cells, did not yield satisfactory results.

Some epithelial cell lines from regenerating mouse liver (72 hours after 70% hepatectomy) have been developed. These cell lines were found to have the capacity to metabolize DMN, 2-acetylaminofluorene (AAF), aflatoxin B_1, cytoxan and urethan, irrespective of their passage numbers in culture. We have used those cell lines as feeder cells (x-irradiated with 500 rads) to metabolize those carcinogens and with C3H 10T 1/2 cells as the target cells. Cytotoxity was our initial criterion to assess the action of those compounds on target cells. Several liver cell lines were tested in this way and most of them were shown to be active. Transformation experiments with C3H 10T 1/2 cells on feeder layers of those liver cells with the above compounds are underway and appear to be highly promising.

Significance to Biomedical Research and the Program of the Institute: The major goal of this project is to develop an assay for carcinogen pre-screening with C3H 10T 1/2 cells. A rapid and convenient system to screen different environmental chemicals for their carcinogenic potential is extremely necessary today. Screening the combination of subthreshold doses of different carcinogens and initiators and promoters is also essential for environmental surveillance since many carcinogens are present in the environment at subthreshold concentrations and may contain substances which act as promoters. In addition, the molecular events in each stage of initiation and promotion are being studied for a better understanding of the process of oncogenesis.

C3H 10T 1/2 cells are easily transformable by polycyclic hydrocarbons, viruses, x-rays, and UV radiation. Thus, the system has the potential to be used to screen environmental carcinogens and promoters. The main drawback in this system is that these cells cannot metabolize the compounds such as DMN, aflatoxin B_1, AAF, etc. Preliminary results show that this difficulty can be overcome by adding a liver microsomal system or by using an established liver cell lines as a feeder layer.

Proposed course: 1) To continue these experiments until a rapid and convenient system to screen different environmental chemicals for their carcinogenic action is perfected, by a) developing a liver microsomal activating system for C3H 10T 1/2 cells; b) developing a cell-mediated activation system by

using regenerating mouse liver cell lines as a feeder layer; c) improving the methods of scoring for transformation by the use of soft agarose. 2) To develop the system as a pre-screen for environmental initiators and promoters. 3) To develop methods for the assay of syncarcinogenesis as a potential pre-screen for combination of weak carcinogens. 4) To study the molecular changes in DNA, RNA and protein synthesis during the initiation and promotion phases of oncogenesis in cell culture for a better understanding of the molecular mechanisms of carcinogenesis.

Date Contract Initiated: September 1, 1976

Current Annual Level: $151,475

SRI INTERNATIONAL (N01-CP-55701)

Title: Development of Detailed Methods and Protocols for Carcinogenic Screening Using Cell Culture Assays - Task IV - Hamster Host Mediated System

Contractor's Project Directors: Dr. Gustave Freeman
Dr. Douglas W. Fodge

Project Officer (NCI): Dr. Virginia C. Dunkel

Objective: To evaluate and determine the usefulness and reliability of cell cultures as assays for detecting chemical carcinogens.

Major Findings: This work has been conducted along three separate but interrelated areas. In one, the status of hamsters that were exposed during pregnancy to various carcinogenic chemicals, to non-carcinogenic analogues, or to solvents for these agents is being monitored. Both the dams and their offspring are being observed. After nearly 2 years, numerous moribund hamsters have been sacrificed and examined for evidence of tumors. Several of the surviving dams that received single injections of B(a)P, 3-MC and DMBA have developed intra-abdominal tumors, whereas no unusual numbers of tumors have developed in other dams. To date, hamsters exposed transplacentally to solvents or to noncarcinogens on day 10 of gestation have developed tumors as often as the carcinogen-exposed offspring, and none have developed striking numbers of tumors. We are also observing dams and offspring exposed on either 12 or day 14 of gestation. Second, the base levels of spontaneous transformation in embryonic cell cultures derived from embryos treated transplacentally are being established. Many early-passage embryonic cell cultures grew in soft agar medium although cells in most cultures had not been exposed to known oncogenic substances. Such spontaneous characteristics occurred at the rate of 1 in 10^5 cells in 15 of 23 untreated cultures, in 2 of 8 solvent-treated cultures and in 8 of 33 organic carcinogen- (and analog-) treated cultures. We are continuing to study the culture conditions that enhance or suppress these spontaneous events. Third, studies were initiated to develop and reproduce the in vitro bioassay system for transformation described by Pienta et al (Int. J. Cancer 19, 642-655 1977). During the past 6 months, primary

cultures, sera, and other components of the culture medium have been screened in order to select the most suitable for use in the studies. Several aspects in these experiments have been varied in order to gain a better understanding of the role of chemical carcinogens in cellular transformation in vitro.

Significance to Biomedical Research and the Program of the Institute: The host mediated in vivo - in vitro cell culture system has not yet proved to be predictable for screening the large number of chemicals that are suspected of being carcinogenic. However, coupled with longterm tumor studies, the system may prove to be important for identifying the mechanisms of tumor induction. These investigations should provide new information about the nature of transplacental oncogenesis, a subject of growing importance and intensive interest.

The in vitro cell culture technique should be useful for screening the large number of chemicals that may have oncogenic effects in man. Moreover, the system may be important for identifying mechanisms of tumor induction by chemicals. This information coupled with the examination of spontaneous transformation in other research, should permit better elaboration of the mechanisms of tumor induction in all animals.

Proposed Course: 1) To investigate whether carcinogen-sensitive cells other than fibroblasts may be detected in animals by allowing offspring of treated mothers to survive indefinitely; 2) continue studies with the host-mediated in vivo - in vitro bioassay described by DiPaolo; 3) to establish the rate of spontaneous transformation in primary cultures of embryonic Syrian hamster cells, and to determine whether the mechanism of "spontaneous" transformation is induced by factors acting in vitro or whether it is the result of selection of preexisting transformed cells; and 4) to test the reproducibility of the in vitro hamster embryo clonal assay for chemical carcinogens according to the method of Pienta et al., and to conduct blind testing of a series of carcinogens and noncarcinogens.

Date Contract Initiated: June 30, 1975

Current Annual Level: $238,571

SRI INTERNATIONAL (N01-CP-65857)

Title: Validation and Utilization of Microbial Mutagenesis System as Prescreens for Chemical Carcinogens

Contractor's Project Director: Dr. Vincent Simmon

Project Officer (NCI): Dr. Virginia C. Dunkel
(NIEHS) Dr. Errol Zeiger

Objectives: To evaluate and validate through collaborative studies in several laboratories microbial mutagenicity and DNA repair assays for their

predictive value in assessing the carcinogenic potential of chemical compounds.

Major Findings: In the initial phase of this project studies were carried out to establish the working protocol, to test the positive control compounds, sodium azide, 9-aminoacridine, 2-nitrofluorene and 2-aminoanthracene, and to prepare and evaluate the mouse, rat and hamster S-9 fractions obtained from non-induced and archlor 1254 induced animals which would be used for metabolic activation.

The first batch of coded compounds for testing was received from the chemical repository in January 1977. Each coded compound is being tested in eight strains of bacteria, namely, Salmonella typhimurium TA 1535, 1537, 1538, 98 and 100 and Escherichia coli WP-2/uvrA$^-$ and W3110/polA$^+$ and p3478/polA$^-$, at seven dose levels both with and without metabolic activation. A base of information is systematically being collected and the decoding and comparative evaluation of the first group of 67 chemicals consisting of known carcinogens and noncarcinogens should be completed late in 1978.

Significance to Biomedical Research and the Program of the Institute: Identification of cancer-causing agents in the environment is a prerequisite for the elimination of such agents from our surroundings. The present study seeks to develop and evaluate rapid, simple and inexpensive microbial procedures to be used as preliminary screens for chemical carcinogens.

Proposed Course: To continue testing a series of coded chemicals consisting of both carcinogens and noncarcinogens for their mutagenicity and DNA-modifying activity.

Date Contract Initiated: September 30, 1976

Current Annual Level: $92,372

SRI INTERNATIONAL (NO1-CP-65854)

Title: Development and Validation of an In Vitro Mammalian Cell Mutagenesis System for Carcinogenesis Screening

Contractor's Project Director: Dr. Ann D. Mitchell

Project Officers (NCI): Dr. Virginia C. Dunkel
(FDA) Mr. Kenneth Palmer

Objective: To evaluate and determine the usefulness and reliability of an in vitro mutagenesis assay system using L5178Y mouse lymphoma cells (TK$^{+/-}$ locus) as a prescreen for potential chemicals carcinogens.

Major Findings: The initial effort on this project has been directed to the development of the L5178Y mouse lymphoma cell mutagenicity assay. The studies to date have proceeded in four phases. In the first phase and prior to testing of chemicals, studies were carried out to demonstrate that the cells would grow

at the reported 4 to 5 doubling rate in 24 hours and could be cloned in soft agar successively at a rate of greater than 75%. In the second phase, six reference chemicals, namely, ethyl methanesulfonate (EMS), hycanthone, methyl methanesulfonate (MMS), N-methyl-n'-nitroso-N-nitrosoguanidine (MNNG), para-rosaniline, and phenanthrene) were tested in the absence of metabolic activation in order to define a suitable protocol. It was determined at this time that changes in assay conditions were required and these included the use of a two-day expression period instead of a three-day expression time and the use of trifluorothymidine (TFT) instead of bromodeoxyuridine (BUdR) as the selective agent for mutant colonies.

Work in the third phase entailed 1) development of metabolic activation methodology using S-9 preparations from the livers of uninduced and Arochlor 1254-induced Fischer rats, and 2) evaluation of ten additional compounds without metabolic activation and with S-9 preparations from both uninduced and induced rats. The chemicals tested in this phase were 2-acetylaminofluorene, benzo(a)pyrene, 7,12-dimethylbenz(a)anthracene, diethylnitrosamine, dimethyl-nitrosamine, diphenylnitrosamine, 3-methylcholanthrene, 2-methyl-4-dimethyl-aminoazobenzene, 2-napthylamine and pyrene.

Phase IV studies are now underway in which all 16 of the above reference chemicals are being retested both with and without metabolic activation.

Significance to Biomedical Research and the Program of the Institute: Since the number of potentially hazardous chemicals that may significantly affect human population groups or the general population far exceeds the capacity of existing long-term animal and carcinogenesis test systems, there is a need for rapid short-term tests such as the mouse lymphoma mutagenesis assay system that may be used in a battery for the initial evaluation of chemicals for possible carcinogenic potential.

Proposed Course: Sufficient confidence with the system has been gained so that in the immediate future approximatley 100 chemicals consisting of both carcinogens and noncarcinogenic analogues will be tested blind to determine and validate the response of the system.

Date Contract Initiated: September 30, 1976

Current Annual Level: $211,213

SRI INTERNATIONAL (NO1-CP-75917)

Title: Detection and Identification of Mutagens in Human Body Fluids

Contractor's Project Director: Dr. Andrew P. Cheung

Project Officers (NCI): Dr. Virginia C. Dunkel
(FDA) Dr. Michael J. Prival

Objective: To determine whether compounds with mutagenic activity can be detected in urine specimens from defined human populations such as cigarette smokers, organic chemists, hospital operating room personnel, and hair dye users, and to isolate and characterize the chemical mutagens.

Major Findings: Recovery experiments with human urine have indicated that nonvolatile organic chemicals of diverse polarity (e.g., 4-amino-2,6-dinitrotoluene, anthracene-2-carboxylic acid, benzidine, 1-chloro-2,3-dibromopropane, N,N-dibutylnitrosamine, N-hydroxybutyl-N-butylnitrosamine, and styrene oxide) can be efficiently $\geq$ 75% recovered) extracted from human urine with an XAD-2 resin column treatment. Based on the mutagenicity assay using *Salmonella typhimurium* strains, a urine specimen from a cigarette smoker has been shown to contain mutagens.

Proposed Course: To develop an efficient and reliable protocol using the XAD-2 column to extract mutagens from human urine specimens. Minimum quantities of various classes of organic chemicals/mutagens will be added to nonmutagenic human urine, and extraction procedure will be performed. At each step, chemical assays and/or the *Salmonella*/microsome test will be performed to determine the extraction efficiency for the various added chemicals. The reliability and efficiency of the extraction protocol will be assessed with urine from rats injected with 2-acetamidofluorene. Mutagenic activities, measured using the *Salmonella*/microsome assay, from unprocessed rat urine and its extracts will be compared. The results will indicate the efficiency and reliability of the extraction protocol.

The accepted protocol will then be applied to assay urine specimens from human test populations for mutagenicity. Urine specimen to which mutagens are added will be treated further for isolation and identification of the mutagens by chemical and biochemical techniques, including high-pressure liquid chromatography, gas-liquid chromatography, and mass and nuclear magnetic resonance spectroscopy.

Significance to Biomedical Research and the Program of the Institute: The objective of this project is to determine whether compounds with mutagenic activity can be detected in body fluids of defined human populations. Considering the high correlation between carcinogenicity and mutagenicity of chemical compounds and that mutagenic metabolites can be detected in the urine of individuals on certain drug regimens, it is possible that mutagenicity assays can be used as a tool for the identification of biologically active substances in human fluids. Combined with fractionation and isolation techniques, this may provide for the eventual identification of compounds which may be contributing to the tumor burden in man.

Date Contract Initiated: August 19, 1977

Current Annual Level: $72,309

UNIVERSITY OF WISCONSIN (N01-CP-85609)

Title: DNA Repair Studies in Cultured Hepatocytes

Contractor's Project Director: Dr. Henry Pitot

Project Officers (NCI): Dr. Virginia C. Dunkel
(FDA): Dr. W. Gary Flamm

Objective: To determine the predictive value of a mammalian cell system using DNA repair as an endpoint in the evaluation of chemical compounds for carcinogenic potential.

Date Contract Initiated: March 29, 1978

Current Annual Level: $59,041

B. TECHNICAL INFORMATION AND RESOURCES BRANCH

October 1, 1977 through September 30, 1978

"Plans, develops and conducts a coordinated technical information and resources program to (1) collect, analyze and disseminate data and information concerning chemical and physical agents which have or may have potential to cause cancer; (2) monitor testing activities in the program and track specific agents under test; (3) identify, collect, evaluate and disseminate scientific findings relevant to carcinogenesis studies, carcinogenesis bioassay and assay technology; and (4) in conjunction with federal and non-federal organizations, establish and evaluate criteria and documentation used to organize and analyze both data and information to increase efficiency and effectiveness of transfer and use."

The Technical Information and Resources Branch was established in July 1977, Dr. Sidney Siegel becoming its Acting Chief. Expansions of the Branch's activities and functions necessitated the recruitment of a Technical Information Specialist and a Computer Programmer.

Since its establishment, major functions of the Branch have included the identification, gathering, collating, storage, analysis and dissemination of data and information generated by or relevant to the Carcinogenesis Bioassay Testing Program. The data and information, for which the group is responsible, is primarily centered around the management conduct and results of bioassay experimentation. This involves screening chemical substances in whole animals or in in vitro systems, analyzing the data and disseminating the scientific findings to those involved in research and administration. Methods of such scientific dissemination have included:

1. The continued but modified support of the secondary journal, Carcinogenesis Abstracts. This publication, which is still under the editorial control and scientific guidance of branch staff, has become a commercial venture for the Franklin Institute Research Laboratories (FIRL). The contract with FIRL carries the proviso that an appropriate number of copies be distributed to a federal mailing list.

2. The PHS-149 publication series, "Survey of Compounds Which Have Been Tested for Carcinogenic Activity", for budgetary restrictions, has not been pursued since the release of the volume covering the 1972-73 scientific literature. A volume covering the 1978 literature is now planned. A consortium of 3 programs, Carcinogenesis Testing, Carcinogenesis Research and the International Cancer Research Data Bank, will contribute to the financing of this project that is to be administered through the branch. This cooperative venture may be a prototype for other intra-NCI program information activities.

3. Three volumes of the IARC monograph series were published and disseminated during 1977.

   A. Volume 13, Subtitled, "Some Miscellaneous Pharmaceutical Substances", includes:

acriflavinium chloride
aurothioglucose
chloroquine
diazepam and oxazepam
dithranol
ethionamide
hycanthone and hycanthone mesylate
8-hydroxyquinoline
metronidazole
niridazole
oxymetholone
phenacetin
phenobarbital and phenobarbital sodium
pronetalol hydrochloride
pyrimethamine

B. Volume 14, Subtitled, "Asbestos", includes:

Chemical and Physical Data
Production, Use, Occurrence and Detection
Biological Data Relevant to the Evaluation of Carcinogenic Risk to Man
Comments on Data Reported and Evaluation
Scope of the Problem Related to Asbestos Exposure

C. Volume 15, Subtitled, "Some Fumigants, the Herbicides 2,4-D and 2,4,5-T, Chlorinated Dibenzodioxins and Miscellaneous Industrial Chemicals", covers the following chemicals:

1,2-bis(chloromethoxy)ethane
1,4-bis(chloromethoxymethyl)benzene
chlorinated dibenzodioxins
copper 8-hydroxyquinoline
2,4-D and esters
1,2-dibromo-3-chloropropane
trans-1,4-dichlorobutene
dihydroxybenzenes
- catechol
- resorcinol
- hydroquinone

dimethoxane
eosin and eosin disodium salt
ethylene dibromide
hexamethylphosphoramide
isopropyl alcohol and isopropyl oils
methyl iodide
para-quinone
succinic anhydride
2,4,5-T and esters
1,2,3-tris(chloromethoxy)propane

Also published and disseminated during 1977 was Information Bulletin #7, "On The Survey of Chemicals Being Tested for Carcinogenicity". The survey gives data received from 98 institutes in 20 countries on a total of 990 chemicals. The objective of this project is to survey ongoing research on long-term carcinogenicity testing throughout the world and by this reduce duplication of research effort and increase scientist-to-scientist communication. The data and information carried includes the chemical being investigated, animal species and strains, routes of exposure, stage of experiment, principal investigator and references to published reports of completed studies.

Branch staff supported the analysis of the operations of the Environmental Mutagen and Environmental Teratogen Information Centers at the Oak Ridge National Laboratories. Both centers are examples of the cooperation possible concerning the administration of data and information activities which have multiple government agency sponsors (NCI, NIEHS, NLM, DOE).

Staff continued as monitors on the Divisional Information System (DIS) to insure that scientific, administrative and fiscal data carried on records describing programs research and resource contracts be complete, accurate and timely to allow the DIS to continue to be used for program planning and analysis.

The Carcinogenesis Bioassay Data System (CBDS) continued its function as Program's major capability to capture, encode, store, analyze and disseminate data and information generated by in vivo carcinogenesis bioassays and thus has been key to the production of issues of the Technical Report Series. Statistical analysis capability, which has been added this year, includes the statistic of beta-binomial distribution.

Except for the inclusion of these statistical routines, the CBDS, as a system, is being kept at a maintainence level for functioning. The reason is that an interagency agreement between the National Cancer Institute and the National Center for Toxicologic Research for the development of a new system to capture toxicology data associated with Carcinogenesis bioassay, has entered its second year and show high promise of data capture in a timely and quality-assured manner. During the last year, under this agreement for system development, NCI and NCTR personnel completed Phase I (Analysis) and part of Phase II (Design). Components of various modules of the Toxicology Data Management System (TDMS) are now detailed enough that negotiations have been ongoing between NCI, NCTR and the Government Services Administration to generate an RFP to allow for the purchase of the hardware and software required to capture bioassay data at the point of experimentation. (Phase III - Implementation)

As the legal mandates of regulatory agencies are more throughly interpreted, it has thus been necessary that the TDMS be reevaluated based on modifications of the scientific experimentation to be performed, captured and analyzed. The design concept of the TDMS has allowed a generalization of the approach to this problem and as such can be considered as a prototype system of the federal government for the management and capture of toxicological experimentation and data.

The following points summarize progress on various of the system's modules:

1. Protocol Analysis Module - Design has been completed and programming begun.

2. Experimental Status Module - Being designed to carry summary information to respond to frequently asked queries about the current operational status of each bioassay.

3. Experimental and Past Experimental Information Modules - Design is complete and implimentation begun on the technology to capture toxicology data at the site of performance.

4. Compound Management System - The data elements required for tracking the selection process have been identified and collection and authentication procedures being established for this information.

Branch staff have been working with Dr. Virginia Dunkel, Coordinator of the In Vitro Program, in the development of a computerized system to manage and capture, store and analyze data from the various in vitro assay screens being evaluated for their ability to detect the carcinogenic potential of chemical substances. A proprietary software package has been purchased for the system and data capture forms developed for the various assay/screens.

The Branch is also presently involved in the development of a management information system which will have the capability to track and inventory any event associated with the bioassay/screens of chemicals and the selection of chemical substances for study, experimental design, allocation to an assay contractor, the acute, subchronic and chronic phases of animal bioassay, pathology.

B. TECHNICAL INFORMATION AND RESOURCES BRANCH
CONTRACT NARRATIVES
October 1, 1977 through September 30, 1978

DOE-NCI INTERAGENCY AGREEMENT (OAK RIDGE NATIONAL LABORATORY) (Y01-CP-20203) (NIEHS)

Title: Environmental Mutagen Information Center (EMIC)

Contractor's Project Director: Mr. J. S. Wassom

Project Officer (NCI): Ms. Joan W. Chase

Objectives: The mission of the Environmental Mutagen Information Center (EMIC) is to collect, organize, and make available information from the field of genetic toxicology. This information may either deal with the testing of chemicals in one of the many available mutagenicity assay systems or deal with studies exploring the mechanisms of mutagenicity. Information collected from the international literature for this data base is processed and stored in computer files and can be selectively retrieved and distributed to the scientific community. Copies of all papers cited in the EMIC data base are maintained by the Center in its own document library.

Major Findings: Approximately 3,800 references were added to the EMIC data base during FY 1978 bringing the total number of references on file to 22,800. These citations have been indexed with Agent/Organism keywords and Chemical Abstracts Service Registry numbers. Correlations with information available in the NCI publication PHS-149 - Survey of Compounds Which Have Been Tested for Carcinogenic Activity were made with compounds in the EMIC Agent Registry. Agents in the EMIC data base have also been cross-referenced to agents appearing in the International Agency for Research on Cancer (IARC) monographs and the Agent Registry of the Environmental Teratology Information Center (ETIC) as well as to chemicals in the Merck Index, the Environmental Protection Agency's Toxic Substance Candidate (TSCA) list and the EPA/NIH Substructure System.

The following table summarizes portions of this information:

No. of chemicals in EMIC Agent Registry	No. of chemicals in Agent Registries cross-referenced to EMIC		No..of chemicals common to both files
8,000	ETIC	3,250	1,177
	PHS-149	3,659	1,250
	IARC (thru Vol. 11)	342	270
	MERCK	9,856	1,884
	TSCA	30,000	3,252

As a result of the wealth of information accumulated in its data holdings and document library, EMIC has been called on repeatedly to provide assistance to a number of groups and agencies concerned with the evaluation of chemicals for carcinogenicity. For example, EMIC supplies mutagenicity information to the NCI Chemical Selection Working Group through SRI International and directly to the IARC for use in preparing its NCI-sponsored monographs.

EMIC's data base is part of the National Library of Medicine's (NLM) TOXLINE system and the Department of Energy's (DOE) RECON system. Anyone with access to either of these files can now query the EMIC data base directly. The chemicals in EMIC's Agent Registry may also be searched via the EPA/NIH Substructure Search System.

Significance to Biomedical Research and the Program of the Institute: Information on chemical mutagenesis is widely dispersed in the international scientific literature and having immediate access to this literature is of importance to workers in the field of carcinogenesis. EMIC's data base provides a unique and readily available source for this information.

The processes of mutagenesis and carcinogenesis are complex and are related in their mechanism(s) of action. In the last few years, as more knowledge has accumulated about the structure and metabolic fate of chemicals with respect to their biological activity, a formal relationship between mutagenicity and carcinogenicity has become apparent. Because of this relationship and the developing use of certain mutagenicity assay systems as prescreens for chemical carcinogens, EMIC's work is acquiring increased significance to cancer research. By having unlimited access to EMIC's data base, the NCI can respond to its own and other requests for information on these and other topics from the area of experimental mutagenesis.

Proposed Course: Due to the correlation between mutagenesis and carcinogenesis and the use of mutagenicity assays as carcinogen prescreens, it seems highly desirable that a data base dealing exclusively with the mutagenicity of chemicals be maintained on a continuing basis.

To maintain a current mutagenicity data base, EMIC will continue to monitor the international scientific literature. All publications which deal with the evaluation of chemicals in the human environment for mutagenicity will be collected and added to the data base. Efforts will continue to index these publications in a manner best suited to meet the needs of researchers and health administrators, and to make this information readily available. Acquisition and processing of literature published before 1967 will be pursued to the extent that time permits.

Inquiries will continue to be made on the use of pattern recognition programs designed to analyze and make comparisons between data selected from mutagenicity and carcinogenicity studies and to correlate these events with chemical structure and/or other chemical features.

Date Contract Initiated: June 30, 1972

Current Annual Level: $110,000

EG&G/MASON RESEARCH INSTITUTE (N01-CP-43257)

Title: Carcinogenesis Bioassay Data System (CBDS) Operational Support

Contractor's Project Director: Dr. Kantilal Patel

Project Officer (NCI): Dr. Kenneth Chu

Objectives: This project links data and information among the bioassay program contractors, the Bioassay Operations Segment, the Carcinogen Bioassay and Program Resources Branch, and the CBDS computer subsystem. The contractor operates a subsystem to accept, log, and process data collection forms submitted by the scientific research organizations participating in the bioassay program. Trained and qualified staff operate this subsystem with functions including: a) controls forms, edits and codes, operates and maintains a microfilmed data base; b) maintains automated files for contract information and chemical information; c) converts histopathologic diagnoses into Systematized Nomenclature of Pathology (SNOP) codes for entry into the CBDS data base; d) provides trained personnel to conduct data seminars and training classes on the use of the CBDS; e) provides statistical analysis routines and preliminary analysis reports for bioassay program use; f) develops Tracor Jitco's Management Information System (MIS); g) maintains and develops *In Vitro* Information System (IVIS).

Major Findings: An operational system to control and process NCI bioassay contractor data from initial data entry to updating the CBDS data base and creating microfiche records for all data processed has been developed and documented in collaboration with NCI and DCRT, NIH. A CBDS chemical file has been developed to maintain current chemical information for all chemicals on bioassay testing. A CBDS contract file has been developed to display administrative, chemical and treatment information for all agents being tested by bioassay contractors. In the area of data processing, over 291,360 data collection forms were processed and entered into the CBDS data base during the current contract period. Data tables have been computerized to display: individual animal pathology, tumor and non-tumor incidence summaries by anatomic site, tissue microscopically examined and tumor incidences, food and weight. Statistical routines have been developed to provide tumor incidence analysis, survival analysis, and control tumor incidence analysis including scatter plots and histograms.

Significance to Biomedical Research and the Program of the Institute: Data collected from bioassay studies are important in formulating public policy pertaining to the continued use of the agents tested. It is important that the results of these studies be properly recorded and analyzed. The CBDS is providing these functions.

Proposed Course: The operational system implemented by the contractor is functioning and is proposed to continue during the development phases of the technical reports on the carcinogenesis bioassay studies.

Date Contract Initiated: June 26, 1972

Current Annual Level: $629,450

EG&G/MASON RESEARCH INSTITUTE (N01-CP-75957)

Title: Data and Information Resources

Contractor's Project Director: Mr. Michael P. O'Flaherty

Project Officer (NCI): Ms. Joan W. Chase

Objectives: To provide the capability of responding to data and information needs of the Carcinogenesis Program, both for quick turn-around and for longer term projects.

Major Findings: No tasks have been performed under this contract.

Proposed Course: The contractor will continue to respond to needs of program staff.

Date Contract Initiated: September 30, 1977

Current Annual Level: $25,000

ENVIRO CONTROL, INC. (N01-CP-75964)

Title: Data and Information Resources

Contractor's Project Director: Mr. Donald M. MacArthur

Project Officer (NCI): Ms. Joan W. Chase

Objectives: To provide the capability of responding to data and information needs of the Carcinogenesis Program, both for quick turn-around and for longer term projects.

Major Findings: Using the basic ordering agreement mechanism, this contractor was able to respond quickly and provide a literature survey in which foreign materials (xenobiotic) such as pesticides and other compounds are discussed relevant to their metabolism.

Proposed Course: The contractor will continue to respond to needs of program staff.

Date Contract Initiated: September 30, 1977

Current Annual Level: $19,646

FEIN-MARQUART ASSOCIATES, INC. (N01-CP-75959)

Title: Data and Information Resources

Contractor's Project Director: Mr. Alvin E. Fein

Project Officer (NCI): Ms. Joan W. Chase

Objectives: To provide the capability of responding to data and information needs of the Carcinogenesis Program, both for quick turn-around and for longer term projects.

Major Findings: The contractor is assigning chemical names to each of the 500-600 compounds in the Carcinogenesis Bioassay Data System and review information presently available and relevant to the structure of chemicals in the testing program under standard bioassay protocol.

Proposed Course: The contractor will continue to respond to needs of program staff.

Date Contract Initiated: September 30, 1977

Current Annual Level: $20,467

FDA-NCI INTERAGENCY AGREEMENT (Y01-CP-70221)

Title: Development and Implementation of a Carcinogenesis Bioassay Management System

Contractor's Project Director: Mr. Larry Lawrence

Project Officer (NCI): Dr. Kenneth Chu

Objectives: The contractor has been analyzing and designing a system appropriate to DCCP bioassay needs. Initially, a determination was made to see if the present NCTR data system might apply and be adapted to this program. Existing modules in the system are:

Experimental Start-Up System (ESS)
Breeding Information System (BIS)
Experimental Information System (EIS)
Post Experimental Information System (PEIS)
Query Language Processor (QLP)

The contractor is determining requirements for a central computer installation with remote terminal configurations, the input requirements for each of the modules, the output requirements, the requirements for an interactive inquiry system, and the requirements for file format. The contractor will also determine the new system's impact on existing bioassay program procedures as well as considering the impact of Good Laboratory Practices (GLP) on the design of the system. Documentation for the system will be developed.

Major Findings: Now that results of the analysis and of the partially completed design phases of the Toxicology Data Management System (TDMS) are available, both organizations are able to commit to acquiring the hardware required for data capture at the sites of bioassay.

Significance to Biomedical Research and the Program of the Institute: The TDMS design has been generalized in order to be able to meet the needs of the rapidly changing science of carcinogenesis bioassay testing. Also, as a result of the emphasis on data capture timeliness and accuracy, the TDMS will be capable of supporting the monitoring and management of bioassay experimentation and thus allow for rapid analysis and dissemination of results.

Proposed Course: Design and implementation of various modules of the TDMS will continue. Installation of data capture hardware is proposed to be accomplished at some sites conducting bioassay during the next year of the interagency agreement.

Date Contract Initiated: April 21, 1977

Current Annual Level: $1,545,000

FRANKLIN INSTITUTES RESEARCH LABORATORIES (NO1-CP-75960)

Title: Data and Information Resources

Contractor's Project Director: Dr. Paul N. Craig

Project Officer (NCI): Ms. Joan W. Chase

Objectives: This project provides the capability of responding to data and information needs of the Carcinogenesis Program, both for quick turn-around and for longer term projects.

Major Findings: By use of a basic ordering agreement, it has been possible to structure information and data-handling tasks (which are defined by the requestor, the project officer and the contractor's project director). The tasks in progress this year include screening the scientific literature for relevant information on selected narrow areas of research, then gathering, collating and evaluating that information.

The tasks re-initiated this year were: (1) "Information on Carcinogenesis Potency Indexes"; (2) "Literature Support for the *In Vitro* Chemical Carcinogenesis Program"; and (3) "Production of an Anatomical Atlas for the European Giant Hamster".

Significance to Biomedical Research and the Program of the Institute: All aspects of scientific research have information needs ranging from searching the current literature to preparing critical reviews which are necessary for the optimal development of a research program. There has been a phenomenal increase of published literature in the past several years including a

proliferation of journals. Since carcinogenesis research encompasses a number of scientific areas, much time and effort can be expended by an individual investigator in pursuing information needs, some of which can be readily handled and others for which the resources are not readily available but are necessary for the proper development of research.

This project provides the resources for satisfying information needs of investigators and science administrators in the Carcinogenesis Program in a timely and effective manner.

Proposed Course: The contractor will continue to respond to the information needs of the Program.

Date Contract Initiated: September 30, 1977

Current Annual Level: $25,000

FRANKLIN INSTITUTE RESEARCH LABORATORIES (N01-CP-75885)

Title: Carcinogenesis Abstracts

Contractor's Project Director: Dr. Bruce H. Kleinstein

Project Officer (NCI): Ms. Joan W. Chase

Objectives: To provide an up-to-date abstract service for carcinogenesis researchers throughout the world in a format designed to provide, under one cover, scientific work in the various disciplines which relate to cancer etiology.

Major Findings: The production of Volume 15 is in progress and is expected to be completed by July 1978. Volumes 15, 16 and 17 will each reference 7,200 articles and contain abstracts and annotations. A Wiswesser Line Notation index and a Chemical Abstracts Registry Number index, in addition to subject and author indexes, are provided in each issue. Close coordination is maintained with NLM and the ICRDB program to make the abstracts and annotations available to the CANCERLIT computerized data base as soon as possible, in advance of publication.

Significance to Biomedical Research and the Program of the Institute: The multidisciplinary approach used in the study of carcinogenesis needs and information exchange medium which gathers the relevant articles under one cover this allows for intellectual cross fertilization among the disciplines to produce a more rapid evaluation and use of information in the study of carcinogenesis.

Proposed Course: To continue the publication for an indefinite period of time.

Date Contract Initiated: August 20, 1977

Current Annual Level: $10,700

ILLINOIS INSTITUTE OF TECHNOLOGY RESEARCH INSTITUTE (NO1-CP-75962)

Title: Data and Information Resources

Contractor's Project Director: Mr. Peter Schipma

Project Officer (NCI): Ms. Joan W. Chase

Objectives: To provide the capability of responding to data and information needs of the Carcinogenesis Program, both for quick turn-around and for longer term projects.

Major Findings: No tasks have been performed under this contract.

Proposed Course: The contractor will continue to respond to needs of program staff.

Date Contract Initiated: September 30, 1977

Current Annual Level: $25,000

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (NO1-CP-45608)

Title: Program on the Evaluation of the Carcinogenic Risk of Chemicals to Humans

Contractor's Project Directors: Dr. Lorenzo Tomatis
Dr. James Huff

Project Officer (NCI): Dr. Sidney Siegel

Objectives: The International Agency for Research on Cancer (IARC), with partial support from the National Cancer Institute of the U. S. (NCI), will continue to pursue actively and accomplish the objectives set forth for the international program on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, which is comprised of two major projects:

Evaluation of the Carcinogenic Risk of Chemicals to Humans:

- collect all available data relevant to the assessment of the carcinogenic risk of chemicals to which humans are exposed;
- analyze and evaluate these data critically with the help of ad hoc working groups of international experts;
- publish and disseminate the evaluative conclusions of these expert panels as IARC Monographs.

Survey of Chemicals Being Tested for Carcinogenicity:

- avoid unnecessary duplication of research;
- increase communication among scientists;
- make a census of research facilities;
- gather, publish and disseminate data on chemicals being tested as IARC Information Bulletins.

Each IARC monograph volume is printed in 4,000 copies and partly distributed free to governments, public health authorities, scientists, etc., and partly via the World Health Organization publications service, and ergo available to anyone upon request, whereas each IARC Information Bulletin is printed in 500 copies and distributed by IARC to all contributors as well as to those with established needs.

Major Findings: To May 1978, sixteen volumes have been published in the IARC Monographs Series on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. More detailed information about the progress and results obtained so far is given in a recent publication (Tomatis, L. et al., Evaluation of the Carcinogenicity of Chemicals: A Review of the IARC Monograph Program, 1971-1977, Cancer Res., 38, April 1978). Further, Volume 17 is in press, Volumes 18 and 19 are in preparation, and meetings for Volumes 20 and 21 have been scheduled.

Of the 368 chemicals evaluated in the first sixteen volumes, 26 chemicals or industrial processes were found, on the basis of epidemiological studies and/or case reports, to be causally associated or strongly suspected of being associated with the occurrence of cancer in humans.

For 221 of the remaining 342 chemicals, some evidence of carcinogenicity in one or more animal species was found.

Information Bulletin No. 7 on the Survey of Chemicals Being Tested for Carcinogenicity appeared in January 1978. This cumulative compilation of ongoing long-term experiments gives data received from 259 investigators doing research at 98 institutes in 20 countries on a total of 990 chemicals. Since Information Bulletin No. 6, 181 research reports have appeared on 180 chemicals. Additionally, of the 905 projects from 70 countries listed in the IARC 1977 Director of On-Going Research in Cancer Epidemiology, about 300 are wholly or partly concerned with 45 chemicals or chemical substances listed in Information Bulletin No. 7.

Bound into each copy of the Information Bulletin No. 7 is a users' evaluation form containing a series of multiple choice questions. So far, 100 responses have been received from a mailing to 250 individuals. A second mailing is planned to obtain results from the other 60 per cent.

Highlighted below are summaries of the relevant events which took place since June 1977 under the joint IARC/NCI international cost-sharing contract agreement:

June 1977: IARC Working Group on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Some Aromatic Amines and Related Nitro Compounds - Hair Dyes, Coloring Agents and Miscellaneous Industrial Chemicals, Lyon, 7-14 June 1977.

August 1977: Volume 15 of the IARC Monographs, containing 18 monographs in 354 pages, was published and distributed - IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man: Some Fumigants, the Herbicides 2,4-D and 2,4,5-T, Chlorinated Dibenzodioxins and Miscellaneous Industrial Chemicals. A list of nearly 900 trade names and synonyms for 2,4-D and 2,4,5-T appears as an appendix.

October 1977: Criteria Meeting for the Evaluation of the Carcinogenic Risk of Chemicals to Humans: IARC/WHO ad hoc working group meeting, Lyon, 3-7 October 1977. Publication: PREAMBLE for the IARC Monograph Program on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, IARC Internal Technical Report No. 77/002, 31 pages.

IARC Working Group on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Some N-Nitroso Compounds and Polychlorinated Biphenyls, Lyon, 10-15 October 1977.

January 1978: Volume 16 of the IARC Monographs, containing 32 monographs in 400 pages, was published and distributed - IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man: Some Aromatic Amines and Related Nitro Compounds - Hair Dyes, Coloring Agents and Miscellaneous Industrial Chemicals.

Joint IARC/NIEHS Working Group on the Coordination of Epidemiological Studies on the Long-Term Hazards of Chlorinated Dibenzodioxins and Chlorinated Dibenzofurans, Lyon, 10-11 January 1978.

Information Bulletin No. 7 on the Survey of Chemicals Being Tested for Carcinogenicity (January 1978, 460 pages) was published and distributed.

February 1978: IARC Working Group on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Some Plastics and Synthetic Elastomers - Monomers, Homopolymers and Copolymers, Lyon, 7-13 February 1978.

April 1978: A special meeting was held with the attempt to select, among chemicals known to have some carcinogenic effect in experimental animals, those for which "strong evidence" of carcinogenicity exists.

Significance to Biomedical Research and the Program of the Institute: The IARC Monographs and the IARC Information Bulletin render valuable assistance to NCI as well as to the international scientific community by contributing to "collecting, analyzing and disseminating data useful for the prevention, diagnosis and treatment of cancer, including the establishment of an international cancer research data bank to collect, catalogue, store and disseminate,

in so far as feasible, the results of cancer research undertaken in any country for the use of any person involved in cancer research in any country" (U. S. National Cancer Act of 1971).

Proposed Course: Monograph program for the coming year to June 1979:
May 1978: Joint IARC/Medizinische Hochschule Hannover planning meeting to consider basic requirements for short-term and long-term carcinogenicity testing, Hannover.

June 1978: IARC Working Group on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Some Halogenated Hydrocarbons, Lyon, 6-13 June 1978.

November 1978: IARC Working Group on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Some Sex Hormones - Natural and Synthetic, Lyon, 21-27 November 1978.

February 1979: IARC Working Group on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Some Sweetening Agents and Some Heavy Metals, Lyon.

Spring 1979: Joint IARC/Medizinische Hochschule Hannover meeting on basic requirements for short-term and long-term carcinogenicity testing, Hannover.

Survey Program

The next survey: In October 1978, the survey questionnnaire for Information Bulletin No. 8 will be mailed to all previous participants as well as to any newly identified investigators/institutes doing long-term chemical carcinogenicity testing.

The next Information Bulletin: Beginning January 1979, all the questionnaire survey data received from the October 1978 mailing will be compiled into Information Bulletin No. 8 which will be finalized and distributed during Spring 1979.

Date Contract Initiated: December 1, 1977

Current Annual Level: $350,256

MITRE CORPORATION/METREK DIVISION (N01-CP-65863)

Title: Preparation of Carcinogenesis Bioassay Reports

Contractor's Project Directors: Dr. Lydia W. Thomas
Dr. Mary Kornreich (September 30, 1977 - May 5, 1978)

Project Officer (NCI): Dr. Thomas P. Cameron

Objectives: The data generated from the compounds tested during the backlog carcinogenesis bioassays are to be compiled, collated, and presented first for review and subsequently as camera-ready copies, to the Data Evaluation Group (DEG) of the Carcinogen Bioassay and Program Resources Branch of NCI. This contract, therefore, requires that Metrek sort through environmental data records, individual laboratory project records, daily laboratory records, etc., to establish and record the design of the experiment. Then the clinical, histopathologic and statistical evaluations as supplied, respectively, by the performing laboratory personnel, examining pathologist(s), and Metrek statisticians are incorporated. The history of animal survival is supplied and from this data and from laboratory animal weight and food consumption records, graphic representations of these are prepared by Metrek for inclusion in the document.

Major Findings: The contract effort described above has been integrated into NCI's masterplan for report writing and is under constant review by the NCI Data Evaluation Working Group.

Significance to Biomedical Research and the Program of the Institute: The availability of these reports will directly impact the ability of regulatory agencies, manufacturers, and the public to draw conclusions pertaining to the continued use or need for further testing of the compounds assayed. Therefore, it is important that the results be accurately recorded in a timely manner. The Metrek Division of The Mitre Corporation is assisting in accomplishing this goal.

Proposed Course: A continuation of the report preparation process, as originally conceived, is contemplated at this time.

Date Contract Initiated: September 30, 1976

Current Annual Level: $95,748

PENNSYLVANIA, UNIVERSITY OF (NO1-CP-75958)

Title: Data and Information Resources

Contractor's Project Director: Dr. David Lefhowitz

Project Officer (NCI): Ms. Joan W. Chase

Objectives: To provide the capability of responding to data and information needs of the Carcinogenesis Program, both for quick turn-around and for longer term projects.

Major Findings: No tasks have been performed under this contract.

Proposed Course: The contractor will continue to respond to needs of program staff.

Date Contract Initiated: September 30, 1977

Current Annual Level: $25,000

SRI INTERNATIONAL (NO1-CP-33285)

Title: A Research Program to Acquire and Analyze Information on Chemicals That Have Impact on Man and His Environment

Contractor's Project Directors: Mr. Arthur A. McGee
Dr. Kirtland E. McCaleb

Project Officer (NCI): Dr. Herman Kraybill

Objectives: It is the objective of this 5-year contract to provide support to the Carcinogenesis Program and the National Cancer Program by determining those chemicals with which man come in contact and making available data and information on their structure, production, distribution and exposure level. In accomplishing this objective, information is developed that assists NCI in (1) choosing those compounds having highest priority for entry into the bioassay testing program, (2) identifying trends in the exposure of man to chemicals in his environment, (3) identifying specific subpopulations who may be at high risk due to chemical exposures, and (4) identifying points at which the information accumulated may be best interfaced with the activities of other agencies, both nationally and internationally.

Major Findings: The major effort during the past year has been the support of the Chemical Selection Working Group (CSWG) by: (1) recommending and documenting the selection of chemicals for bioassay; (2) helping to improve the selection process; and (3) supporting the operation of the CSWG on a daily basis via a three-member liaison group established in Bethesda, Maryland.

SRI continued to support the production of the Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man being produced by the International Agency for Research on Cancer (IARC). SRI assists IARC in the selection of chemicals for study and provides chemical and physical data, production, use, occurrence, and analysis information on selected chemicals.

Work has continued on the expasion of the exposure data bank. Exposure categories under development are agricultural chemicals, adhesives and sealants, and occupational exposure.

Efforts have continued to complete a thorough documentation of the data processing system.

Through this contract, supported by NLM funding, SRI has continued to provide information to NLM's Toxicology Information Program.

Significance to Biomedical Research and the Program of the Institute: This study provides information on many chemicals of interest both to the Carcinogenesis Program and to other programs concerned with the impact of chemicals on man.

Date Contract Initiated: May, 1973

Current Annual Level: $709,783

SYSTEM SCIENCES, INC. (NO1-CP-75963)

Title: Data and Information Resources

Contractor's Project Director: Mr. Joseph Rohm

Project Officer (NCI): Ms. Joan W. Chase

Objectives: To provide the capability of responding to data and information needs of the Carcinogenesis Program, both for quick turn-around and for longer term projects.

Major Findings: No tasks have been performed under this contract.

Proposed Course: The contractor will continue to respond to needs of program staff.

Date Contract Initiated: September 30, 1977

Current Annual Level: $25,000

TRACOR JITCO, INC. (NO1-CP-75961)

Title: Data and Information Resources

Contractor's Project Director: Mr. Randall E. Huffman

Project Officer (NCI): Ms. Joan W. Chase

Objectives: To provide the capability of responding to data and information needs of the Carcinogenesis Program, both for quick turn-around and for longer term projects.

Major Findings: In accordance with the objectives of a basic ordering agreement, this contractor was able, within 24 hours, to supply copies of current literature on hair dyes for the Congressional hearings on the budget. The contractor has also completed a literature search for all short-term

tests in whole animal (in vivo) systems involving carcinogenesis and mutagenesis endpoints. Ongoing tasks such as literature searches and reprint reproduction for the Toxicology Branch, the topics to be specified by individual investigators and administrators have also been awarded to this contractor.

Proposed Course: The contractor will continue to respond to needs of program staff.

Date Contract Initiated: September 30, 1977

Current Annual Level: $22,741

C. TOXICOLOGY BRANCH

October 1, 1977 through September 30, 1978

Plans, develops and conducts a research program to (1) develop experimental designs for carcinogenicity testing in animals; (2) monitor the quality of test performance and in collaboration with other related programs in the Institute to analyze and evaluate final data for determination of carcinogenicity; (3) develop bioassay methodologies to permit accurate identification of carcinogenic agents in animals, and their relevance to potential human hazards; (4) study possible mechanisms of carcinogenicity through toxicological methods; and (5) develop and standardize models for carcinogenesis testing.

The Toxicology Branch was established in January 1977 as a component of the Carcinogenesis Testing Program (CGT) and derived many of its initial personnel from its predecessor, the Carcinogen Bioassay and Program Resources Branch (CBPRB). Current staff consists of Dr. Cipriano Cueto, Jr., Chief and Toxicologist; Dr. Kenneth C. Chu, Chemist; Dr. Harry A. Milman, Pharmacologist; Dr. Thomas W. Orme, Microbiologist; and Dr. Carrie E. Whitmire, Pharmacologist/ Toxicologist.

The Branch provides support for the entire Carcinogenesis Testing Program by managing and monitoring specific bioassay contracts and by directing the scientific activities of the NCI Experimental Design Group (EDG) and the NCI Data Evaluation Group (DEG). Although the Branch is not formally divided into sections, its activities are related to three main areas; methodology development and management, data evaluation, and risk assessment. Experimentally, the efforts of Branch personnel are focused on the support of in vivo carcinogen bioassays and on the development of new approaches to data analysis.

(1) Carcinogen Bioassay - This year has seen the accomplishment of a major commitment to this Program area: The reporting of over 200 chemicals which have been tested in the Program. This involved the collating of data and information report writing, evaluation of the report, its publication and dissemination. In the process, the NCI DEG evolved as a nationally recognized group for assessing evidence for carcinogenicity from animal studies.

General aspects of the Branch activities relate to the development of improved methodology and improved predictability of carcinogen bioassay in terms of various test species.

Specific aspects of the Branch activities, in cooperation with other elements of the Program, relate to the (a) establishment of logistical capabilities for testing; (b) acquisition, characterization, and purification of these agents; (c) establishment of testing protocols through the NCI EDG: (d) implementation of the appropriate bioassay tests; (e) monitoring test progress and performance; (f) analysis and evaluation of the test results; (g) report writing; (h) evaluation of reports through the NCI DEG; and (i) devision on further action required concerning the tested agents. The Branch activities encompass the monitoring of a number of contracts dealing with structure-activity relations

as a method for predicting carcinogenicity. The members of the Branch provide direction and management to those studies as well as expertise and support in the development of the necessary resources for animals, chemicals and information systems. The main categories of compounds on test are pesticides and related chemicals, drugs and cosmetics, chemical intermediates and reagents, dyes and related chemicals, solvents, food or food additives, and rubber and paint chemicals. Procedures for the selection of chemicals for carcinogenesis bioassay were modified during the year in collaboration with other Program staff and with the Prime Contractor. Emphasis is now placed on review of classes of chemicals rather than individual compounds. Information resources developed by the Branch in conjunction with the Technical Information and Resources Branch have provided the required basis for decisions on testing.

The NCI EDG reviews the objectives for bioassays to be assigned to contractors and gives guidance in the development of specific protocols. The EDG presents its findings to a corresponding subgroup of the Clearinghouse on Environmental Carcinogens. The EDG is administratively and technically responsible directly to the Chief of the Branch. The Group is charged with the following responsibilities:

(1) Establish basic objectives of the test and data elements required for each chemical considered.

(2) Establish specific experimental design to obtain the required data and provide a written outline of the protocol recommended.

(3) Maintain close interaction with DEG and identify general objectives and problem areas in experimental design and formulate recommendations.

The DEG was established in August 1976 to meet the objectives of efficiently reporting results of carcinogenesis bioassays while maintaining a rigorous and systematic evaluation of the experimental data. The DEG presents its technical findings to a corresponding subgroup of the Clearinghouse on Environmental Carcinogens. However, the DEG is administratively and technically directly responsible to the Chief of the Branch. The Group is charged with the following responsibilities:

(1) Establish basic data requirements for specific type of reports.

(2) Establish criteria for data review and evaluation.

(3) Review and evaluate collated data as to its biological and statistical significance in the assessment of the possible carcinogenicity of a specific chemical to experimental animals.

(4) Formulate recommendations as to data needs, limitation of interpretation, and nature of report to be written.

Maximum tolerated doses (MTD) for chemicals going on test are estimated through review and evaluation of subchronic data by toxicologists from the Branch, Prime Contractor, and subcontractors. The objective is to set MTDs that result in good survival of treated experimental animals without the need to change doses during the progress of the chronic treatment.

One of the most pressing concerns in the Testing Program is the need to fully document and make available the details of the methodology, test performance, and results of each chemical bioassay. While usual reports in the literature or at conferences will still be encouraged, the NCI Carcinogenesis Technical Report Series has been established to provide the necessary medium for all public disclosure of bioassay detailed reports. Contracts with Tracor Jitco, Inc., and the Mitre Corporation provide most of the effort in collating data and preparing the technical reports. Branch staff personnel assure input of data from the CBDS. The critical evaluation and review of technical reports now represents a major undertaking of the Branch. Procedures have been developed for the release of technical reports in such a way that all interested parties, Government and private, receive the results systematically. The Office of Cancer Communications (OCC) announces the availability of the reports in a press release, along with an announcement in the Federal Register. These procedures were approved by NCI and DHEW.

This year, information requests have greatly increased, partly because of increased knowledge of the chemicals on test. The Branch has provided professional advice to regulatory agencies and other branches of the Government on the interpretation of carcinogen bioassays conducted outside the Carcinogenesis Testing Program or interpretation of the implications of an NCI bioassay on chemicals used or regulated by other branches of the Government.

C. TOXICOLOGY BRANCH
CONTRACT NARRATIVES
October 1, 1977 through September 30, 1978

CALIFORNIA, UNIVERSITY OF (at Santa Cruz) (N01-CP-75816)

Title: Computer-Aided Prediction of Metabolites for Carcinogenicity Studies

Contractor's Project Director: Dr. W. Todd Wipke

Project Officers (NCI): Dr. Kenneth Chu
Dr. Sidney Siegel

Objectives: The primary objective of this investigation is to establish a computer program by which a biochemist or metabolism expert can explore the metabolites of a chemical compound. It is expected that the user of the program will be experienced in metabolism or carcinogenesis, but not in the use of computers, and that the user may be geographically remote from the host computer. The goal is to methodically generate all plausible metabolites of a xenobiotic compound so that they may be evaluated to determine if the compound might be carcinogenic and should be scheduled for testing.

Major Findings: Using technology derived from the field of computer-assisted design of organic synthesis, a prototype program called XENO has been developed which demonstrates that this approach is feasible. XENO runs on a PDP-10 computer, using either a GT40 graphics terminal or a teletype for input of the compound for study. XENO includes a large library of metabolic transformations coded in the ALCHEM language, which are both human and machine readable. As each transform is applied, a metabolite is generated, displayed to the user, and stored in a "metabolism tree." The user directs XENO regarding which metabolites to metabolize next. XENO analyses have been compared to experimental results in the literature and have been evaluated by metabolism experts. These evaluations verified that the library of transforms is incomplete, but in some of the cases studied, XENO not only reproduced what is known in the literature, but also proposed some plausible pathways which are not known in the literature.

Significance to Biomedical Research and the Program of the Institute: This project will ultimately lead to a tool for exploring the domain of plausible metabolites, which will be useful not only for carcinogenesis studies, but also for pharmacology, toxicity, and environmental studies. It will be used to guide researchers to know what kind of metabolites to look for in the laboratory and will allow testing various theories of metabolism as well. Clearly, it is relevant in drug design as well, since metabolism is a key factor there too. A second stage of the project will compare each metabolite generated to a library of known carcinogens and will then mark those metabolites which are identical or "similar" to known carcinogens.

Date Contract Initiated: March 8, 1977

Current Annual Level: $37,485

CASE WESTERN RESERVE UNIVERSITY (N01-CP-75927)

Title: Use of Physicochemical Parameters in Obtaining Structure-Activity Relationships with Potentially Cancer Related Endpoints

Contractor's Project Director: Dr. A. J. Hopfinger

Project Officer (NCI): Dr. Kenneth Chu

Objectives: The major objective of this project is to be able to compute physicochemical features of molecules which correlate with carcinogenic endpoints. A secondary objective is to develop the mathematical techniques to establish the correlations between structure and activity. The goal of the work is to use the structure-activity correlation equations to predict the carcinogenic potential of a compound.

Major Findings: A relationship has been established between carcinogenic potency with aqueous and lipid solvation-free energy for a set of nitrosamines. This type of analysis is also being repeated on the NSF health index data base. An action model has been suggested on the basis of the correlation. Aziridinium nitrogen mustard has been interacted with DNA bases using molecular orbital modeling calculations. Specific alkylation reactions were determined and probable sites of alkylation projected. Two observed minor alkylation sites, N-3 of adenine and cytosine, were not predicted to undergo alkylation unless a conformational change occurs in B-form, double-stranded DNA. Both minor alkylation sites miscode in vitro. Thus, the carcinogenic specificity through DNA alkylation may be controlled by local conformational changes. Molecular mechanics calculations of prealkylation sites for carcinogens with B-form DNA suggest little specificity between base site and the carcinogen. Thus, a tentative conclusion has been reached that chemical reactivity holds the key to specificity. Various epoxidized forms of isomeric benzo(a)pyrene are currently being analyzed.

Significance to Biomedical Research and the Program of the Institute: This project is important to biomedical research in general and the Carcinogenesis Program in particular in two ways. First, physicochemical descriptors obtained from linear-free energy (LFE) models and molecular mechanics calculations can be used as correlation indices to model and predict gross carcinogenic features. The quantitative structure-activity relationship (QSAR) analyses of some cyclic nitrosamines and the NSF health hazard index data base are examples of this application. Secondly, the more extensive quantum mechanics calculations, as well as many molecular mechanics calculations, can be applied to small, structurally detailed compound sets to model possible mechanisms of carcinogenic action. The intermolecular physical and reaction calculations described of protonated aziridine models of nitrogen mustard with DNA are examples of this class of investigations.

Date Contract Initiated: September 1, 1977

Current Annual Level: $98,678

HOWARD UNIVERSITY (N01-CP-33266)

Title: Chemical and Biological Investigation of Potential Carcinogens from Plants

Contractor's Project Directors: Dr. Govind J. Kapadia
Dr. S. N. Pradhan

Project Officer (NCI): Dr. Elizabeth Weisburger

Objectives: The overall objectives of this project are: (1) to prepare purified fractions from certain herbs that are used habitually in Curacao and South Carolina and isolate and characterize crystalline compounds from these fractions; (2) to test each fraction and compound for carcinogenicity in experimental animals and identify substances responsible for the carcinogenic effects; (3) to extend such chemical and bioassay studies to plant species related to the above, as well as to other plants that are suspected of possessing carcinogenic activity; and (4) to elucidate the chemical structure of any carcinogenic substance that may be isolated. During the current project period, the objective was to conduct acute, subacute, and subchronic toxicological experiments using esophageal instillation procedures for four plant materials that were earlier found to be tumorigenic following their repeated subcutaneous injections, with a view to selecting a dose for their long-term chronic investigation.

Major Findings: This project is a continuation of an earlier investigation which was developed in an attempt to correlate the high incidence of esophageal carcinoma in natives of certain places with their habit of using herbaceous folk remedies. In earlier studies, 34 samples, comprised of extracts and fractions of 21 plants, were bioassayed for carcinogenicity by subcutaneously administering the products to NIH black rats. Among these plants, 12 tannin-rich plants were found to be tumorigenic, the extract and tannin fraction of *Acacia villosa* roots (AV) being the most potent, causing tumor in the shortest time in over 90% of the treated animals. The other most significant part of the results was that the tannin fraction, *Camellia sinensis* (AT), which is commonly consumed as a beverage, was found to be carcinogenic, causing tumor formation in 70% of the experimental animals. Two other plant materials, *Sassafras albidum* root bark (SA) and *Diospyros virginiana* leaf (DVL), which are also used as beverages, but are not rich in tannins, were found to induce tumors in the experimental animals. The safrole-free extract of SA bark and the aqueous extract of DVL were tumorigenic in 66% and 56% of the subcutaneously treated animals, respectively. For the present studies, Fischer rats were administered by esophageal instillation total aqueous extracts of AV, AT, DVL, and safrole-free extract of SA. These materials, with their effective dose (mg.) were: AV - 2, AT - 12, DVL - 15, and SA - 15. In the acute experiment, each material was given to five male and five female rats by single instillation at five dose levels in geometric proportion, the lowest dose being ten times the subcutaneous tumorigenic dose. On the basis of mortality, decrease in body weight and food intake, and character of stool observed over two weeks, the approximate maximum tolerated dose (MTD) in mg. was selected to be: AT - 120, AV - 80, DVL - 1200, and SA - 1200. In the subacute experiment, five doses of each material were selected on the basis of the MTD in the acute

experiment for repeated instillation daily for five days a week for two weeks. Based on the criteria - particularly on body-weight change - in the acute experiment, the approximate MTD (mg.) for the materials was estimated as follows: AT - 12-24, AV - 8-32, DVL - 240-480, and SA - 60-120 (depending on the sex). However, no clear-cut estimation of MTD was possible.

In the subacute experiment, five doses of each material were chosen around the MTD from the subacute experiment for repeated instillation daily for five days a week for 13 weeks each in ten male and ten female rats. Attempts were made to determine the MTD on the basis of weight change only, because changes in other parameters were not meaningful. Since weight increase (rather than decrease) was observed in almost all the groups with respect to the saline control group, MTD determination for the plant material became difficult. However, with respect to data on its lowest dose group, the male rats decreased in body weight with varying numbers of higher doses, but no clear dose-response relation was evident. On the other hand, in the female groups, weight increase was observed when compared with the data from the saline, as well as low-dose treated groups. Weight increase was observed in all high-dose groups, except in SA group. No specific histological change ascribable to drug effect was observed in any rat group.

Chemical analyses of each of these four plants were carried out, and characterization studies on several isolated products were performed. Included among these products were known compounds not hitherto reported in these plants, as well as unknown products which are being further studied.

Significance to Biomedical Research and the Program of the Institute: This project is directly related to the major objective of the Carcinogenesis Program, i.e., the identification of unknown carcinogens in natural plant materials. This project should serve as the ultimate check to ascertain the carcinogenicity of materials implicated by epidemiological studies on the island of Curacao and in South Carolina. Information gained from this study could conceivably lead to definitive studies that should be conducted in other high-incidence areas to identify the carcinogens involved.

Proposed Course: It is proposed to set up short-term bioassays with a view to monitoring fractionation and isolation of active compounds from plant extracts hitherto found to be carcinogenic. The isolated compounds will be characterized. Based on the results of the short-term bioassays, chronic studies will be undertaken following acute, subacute, and subchronic toxicological experiments.

Date Contract Initiated: June 25, 1971

Current Annual Level: $132,234

JOHNS HOPKINS UNIVERSITY (N01-CP-75929)

Title: The Use of Physicochemical Parameters in Obtaining Structure-Activity Relationships with Potentially Cancer Related Endpoints

Contractor's Project Director: Dr. Joyce J. Kaufman

Project Officer (NCI): Dr. Kenneth Chu

Objectives: The ultimate objective of the research is to delineate the molecular basis of chemical carcinogenesis. The present project has as its goal to determine mathematically the combination of theoretical molecular properties and experimental physicochemical properties that influence the known potentially cancer related endpoint of compounds, both to gain insight into the mechanism of chemical carcinogenesis and to develop capabilities which could be applied to predicting effects of untested compounds in systems.

Major Findings: Quantum chemical calculations have been carried out for carcinogenic polycyclic aromatic hydrocarbons and their metabolites and for the attack of an ultimate carcinogen, CH_3, on guanine. These calculations were carried out with ab-initio programs which incorporate two desirable options: ab-initio effective core model potentials (MODPOT) and a charge conserving integral approximation (VRDDO - variable retention of diatomic differential overlap), especially effective for spatially extended systems. Among the systems studied were benzo(a)pyrene and its oxides, dihydrodiols and diolepoxide, dimethylbenzanthracene derivatives, and methylcholanthrene.

Since the polycyclic aromatic hydrocarbons are quite large molecules and their derivatives are closely related, a new MERGE program was written in order to calculate the new integrals only and to reuse all the integrals of a common skeletal fragment. The MERGE program saves orders of magnitude of computer time when studying systems of such large molecules and allows the completion of electronic wave functions of the carcinogens, their metabolites, and the attack of ultimate carcinogens on DNA constituents.

Electrostatic molecular potential contour maps have been generated around the carcinogenic polycyclic aromatic hydrocarbons and their metabolites. These maps give insight into the reactivities toward metabolism and electrophilicity of the ultimate carcinogenic metabolites.

Significance to Biomedical Research and the Program of the Institute: The computational capability to calculate big molecules reliably yet tractably is the necessary foundation for calculating theoretical molecular properties and reaction pathways to delineate the mechanism of chemical carcinogenesis. Once a better understanding is gained of the mechanism of chemical carcinogenesis, it should be possible to predict potential carcinogenicities of untested compounds and to devise strategies to prevent or ameliorate chemical carcinogenesis.

Furthermore, since the computational methods are completely general, they are applicable to biomedical research problems where the wish is to determine the electronic wave functions of the constituent molecules.

Proposed Course: To continue to investigate the molecular properties of the carcinogens and their metabolites, to initiate theoretical studies on the actual mechanisms of the processes by which these take place, to calculate the interactions of ultimate carcinogens with DNA constituents, and to use appropriate

mathematical techniques to determine the combination of theoretical and experimental properties which govern chemical carcinogenesis.

Date Contract Initiated: September 7, 1977

Current Annual Level: $81,009

NCI-FREDERICK CANCER RESEARCH CENTER (Litton Bionetics, Inc.) (NO1-CP-75380)

Project #8

Title: Bioassay Research

Contractor's Project Director: Dr. Donald Creasia

Project Officer (NCI): Dr. Richard A. Griesemer

Objectives: Frederick Cancer Research Center (FCRC) conducts a limited number of bioassays of chemicals chosen by the NCI Carcinogenesis Testing Program (CGT) and of nitrosamines chosen by the Director of the Chemical Carcinogenesis Program at FCRC.

Major Findings: Evaluation of 21 chemicals on which bioassays have been performed in the FCRC Program is in progress. Evidence for the carcinogenicity of azobenzene, 2,4-toluenediamine, and nitrosodiphenylamine has been gathered. Bioassays of six additional compounds have been started. These compounds are randox, telone, cyclohexanone, styrene oxide, benzyl chloride, and methapyrilene. Dose-response studies using several nitrosamines in rats have begun, with the object of determining the variations in responses, which will have a bearing on approaches to risk extrapolation from the results of bioassays in rodents.

Significance to Biomedical Research and the Program of the Institute: The purpose of this contract is to determine the carcinogenicity of assigned environmental chemicals in rodents.

Proposed Course: Bioassays assigned by the Program to FCRC will be implemented according to designs determined by the NCI Experimental Design Group (EDG).

Date Contract Initiated: September 26, 1977

Contract Annual Level: $761,398

NEBRASKA, UNIVERSITY OF (EPPLEY INSTITUTE FOR RESEARCH IN CANCER) (N01-CP-33278)

Title: A Resource for Carcinogenesis Bioassays and Related Research

Contractor's Project Director: Dr. David B. Clayson

Project Officers (NCI): Dr. David Longfellow
Dr. Thomas Orme
Dr. Carl Smith

Contract narrative is reported under the Interdisciplinary Programs and Resources Operational Unit of the Carcinogenesis Research Program.

Date Contract Initiated: March 19, 1968

Current Annual Level (Carcinogenesis Testing Program): $1,407,551

PENNSYLVANIA STATE UNIVERSITY (N01-CP-75926)

Title: The Use of Physicochemical Parameters in Obtaining Structure-Activity Relationships with Potentially Cancer Related Endpoints

Contractor's Project Director: Dr. Peter C. Jurs

Project Officer (NCI): Dr. Kenneth Chu

Objectives: To correlate structurally diverse compounds with their known carcinogenic potential using chemical structure information handling and pattern recognition methods to search for invariant properties of the known carcinogens. To develop predictive methods for determining the effects of new chemicals in living systems. To determine the structural factors that correlate with carcinogenic activity. These objectives are being approached using the ADAPT computer software system.

Major Findings: Three sets of data have been studied: a set of 209 heterogeneous compounds taken from the literature and including more than ten structural classes, a set of over 400 polycyclic aromatic hydrocarbons, and a set of over 100 N-nitroso compounds. The heterogeneous data set has been studied thoroughly. For each compound of the 209, a set of more than 30 computer-generated descriptors were developed. These included fragments (atom and bond counts and molecular weight), substructure descriptors (noting the number of occurrences of a particular, explicitly defined substructure), environment descriptors (which code the surroundings of a particular substructure of interest), geometric descriptors (to code the size and shape of molecules), molecular connectivity descriptors (to code the overall branching and thus, the lipid/aqueous phase partition), and physicochemical descriptors. A variety of pattern recognition algorithms have been used to analyze the data thus generated. The best result for the 209 compounds is obtained with a Bayes classifier, which implements a quadratic decision surface and correctly classifies 96% of the data (eight misses out of 209 compounds split into 130 carcinogens and 79 noncarcinogens). The best linear decision surface obtained a classification success rate of 90% (20 wrong out of 209 compounds). Further studies with the 209 compounds and with the other data sets are proceeding.

Significance to Biomedical Research and the Program of the Institute: This project will begin to explore the applicability of modern computer-based methods to the study of structure-activity relations of carcinogens. A search is being made for molecular structure features that correlate with carcinogenic potential. The identification of adequately representative descriptor sets and the generation of successful classifiers will produce insights into the nature of chemical carcinogens. Computer-based prescreens will result that can be used for prediction of carcinogenic potential of untested compounds.

Date Contract Initiated: September 1, 1977

Current Annual Level: $45,453

SRI INTERNATIONAL (N01-CP-75930)

Title: The Use of Physicochemical Parameters in Obtaining Structure-Activity Relationships with Potentially Cancer Related Endpoints

Contractor's Project Director: Dr. Howard L. Johnson

Project Officer (NCI): Dr. Kenneth Chu

Objectives: This project is aimed at the development of statistically defined mathematical relationships between chemical structure (and associated quantitative physicochemical properties) and mutagenicity. The purpose of developing such quantitative structure-activity relationships (QSAR) is: (1) to provide a predictive tool for estimating the probable carcinogenicity hazards of untested environmental chemicals (prioritization for experimental test programs), and (2) to discern relationships that may be suggestive of fundamental mechanisms of mutagenicity and carcinogenicity.

Major Findings: Efforts thus far have been concentrated on a large mutagenicity data base for chemicals which are all classed as aromatic amines. Using multiple regression analysis techniques, a mathematical relationship has been defined for 53 compounds, based on mutagenicity data for S. typhimurium TA1538. Statistically, the correlation accounts for more than 83% of the activity variance, based on steric and electronic effects of the aromatic nuclei and substituents. The correlation is consistent with current mechanistic knowledge and has been moderately successful in tests of its predictive utility when applied to new data. In collaboration with Dr. Gilda Loew of Stanford University, quantum chemical parameters have been calculated to explain the activity variance among compounds in the original data set, which could not be accommodated well in the multiple regression correlation. Two manuscripts describing these approaches are currently in preparation.

Significance to Biomedical Research and the Program of the Institute: This research will yield scientific correlation data relevant to the areas of mutagenicity/carcinogenicity mechanisms and will impact on the environmental concerns of the National Cancer Institute (NCI) and other organizations such as EPA, NIOSH, and FDA. An expected early benefit of the study is the provision of an empirical, but mechanistically significant and statistically defined methodology, for the major Carcinogenesis Program goal of identifying chemical causes of cancer.

Proposed Course: Current research centers on expansion of the data base applicability of regression correlations via inclusion of quantum chemical parameters along with linear-free energy and steric parameters. Applications in the area of polycyclic aromatic hydrocarbons are contemplated, in part because of probable mechanistic overlap between these and aromatic amines which can be considered to be members of both classes.

Date Contract Initiated: September 1, 1977

Current Annual Level: $101,153

STANFORD UNIVERSITY MEDICAL CENTER (NO1-CP-75928)

Title: The Use of Physicochemical Parameters in Obtaining Structure-Activity Relationships with Potentially Cancer Related Endpoints

Contractor's Project Director: Dr. Gilda Loew

Project Officer (NCI): Dr. Kenneth Chu

Objectives: The primary objectives of the investigation are to identify and calculate mechanistically-meaningful molecular parameters which will correlate to the best available indicators of carcinogenic potencies of chemical carcinogens and to use these parameters to devise a routine screening procedure for untested coumpounds. To this end, enzymatic and nonenzymatic transformations of classes of chemical carcinogens leading to activation and detoxification are being studied, ultimate carcinogens characterized, and the interaction of ultimate carcinogens with nucleic acids described. From these mechanistic studies, parameters are being selected and calculated which should correlate with observed mutagenic and carcinogenic potencies in a series of related compounds.

The basic techniques used are those of quantum chemistry embodied in large-scale computer programs which calculate molecular conformations, electronic structure, reactivity parameters, and intermolecular interactions.

Major Findings: Work done during this period has focused primarily on two classes of indirectly acting carcinogens: polycyclic aromatic hydrocarbons and amines. In addition, in preparation for modeling interactions of ultimate carcinogens, a new perturbation methodology has been tested, and the interactions of DNA ethidium bromide and actinomycin-D, two known mutagens and intercalaters, have been studied.

A significant body of data has been generated for a series of 15 PAH with varying mutagenic and carcinogenic potencies. All of these compounds require multiple transformation to active carcinogens. Parameters were calculated for several of these transformations. Electronic properties relevant to the efficacy of parent compounds as substrates for P-450 were calculated and successfully related to the known distributions of metabolites for these compounds. Activation of proximate bay region bonds in diols to epoxidation was found to occur equally well in carcinogenic and noncarcinogenic compounds and not to be a significant step in determining carcinogenic activity.

Stabilities were calculated for 15 carbonium ions of bay region epoxide diols, thought to be the ultimate carcinogens of polycyclic aromatic hydrocarbons, and found to correlate with observed carcinogenic potencies.

Reaction pathways for rearrangement and hydrolysis reactions of arene epoxides were studied and the origin of the "NIH shift" explained. Reaction pathways for rearrangements and hydrolysis of arene diol epoxides were also studied, and their greater tendency to attack target tissue nucleophiles, compared to epoxides, was rationalized.

A significant body of data has also been generated for a series of eight polycyclic aromatic amines and a series of 15 aniline derivatives. Reaction pathway studies for N-hydroxylation of amines by cytochrome P-450 were studied. The results appear to rule out insertion and favor a hydrogen abstraction or addition-rearrangement enzymatic mechanism for transfer of an electrophilic oxygen to the amine.

For the parent compounds, electronic reactivity parameters were calculated which relate to their relative extent of N-hydroxylation ring epoxidation and ring hydroxylation. These parameters correlated well with known mutagenic

potencies of pairs of isomers of polycyclic aromatic amines and with activity of the 15 aniline derivatives. Reasons for the inactivity of chloroanilines became apparent: inactivation of N-hydroxylation, thus favoring detoxifying ring hydroxylation.

Results for nitroaniline derivatives also indicated that nitro groups would impede N-hydroxylation. Those nitroaniline compounds which are mutagenic most likely proceed by transformation of the nitro group itself.

Methodological advances were made in two directions. The success of the ethidium-DNA studies in accounting for the observed specificity of ethidium binding justifies judicious use of semiempirical molecular orbital methods to characterize intermolecular interactions. A quantum mechanical perturbation formulation of intermolecular interactions, including overlap, and built on semiempirical methods, was also tested. The results compared favorably with more rigorous ab-initio approaches and point to the continued usefulness of this more efficient method to characterize ultimate carcinogen-DNA interactions.

Significance to Biomedical Research and the Program of the Institute: This project will yield further insight into mechanisms of important processes involved in chemical carcinogenesis and will allow the selection and calculation of meaningful molecular parameters which should correlate to this activity. Studies of the mechanisms of enzymatic and nonenzymatic transformations leading to activation and detoxification of different classes of chemical carcinogens will provide useful information about these mechanisms and aid in selecting relevant molecular properties related to transformations. Such properties should contribute to the manifest activity of parent compounds. Studies of the interaction of proposed ultimate carcinogens of different classes of compounds with nucleic acid sites thought crucial to mutation and initiation of carcinogenesis will help validate proposed mechanisms and provide additional parameters related to activity.

A set of indices which are relevant to both transformations and interactions with nucleic acid should prove useful in correlations to known carcinogenic activity of closely relative compounds. If so, these indices can be used to establish a screening methodology for untested compounds that will save countless manyears of effort.

Proposed Course: During the remaining four months of this contract, it is proposed to complete work on reaction pathway studies of P-450 epoxidations and hydroxylations, on nonenzymatic rearrangements of intermediates of polycyclic aromatic hydrocarbons, on correlations of indices related to transformations with metabolic products and mutagenic potencies, on characterizations of ultimate carcinogens of polycyclic aromatic hydrocarbons and amines, and on preparation of additional manuscripts for publication.

Date Contract Initiated: September 1, 1977

Current Annual Level: $82,747

MPLE UNIVERSITY (N01-CP-43271)

Title: UV Photocarcinogenesis: Effects of Depletion of Atmospheric Ozone

Contractor's Project Director: Dr. P. D. Forbes

Project Officer (NCI): Dr. Henry Hennings

Objectives: The primary objective of the investigation is to identify and quantitatively assess the relationship between changes in the "effective thickness" of stratospheric ozone and the development of solar damage to the skin, particularly carcinogenesis. The experiments are designed to give data based on several effective thicknesses of ozone, the principal biologic endpoint being carcinogenesis in the skin of hairless mice. A xenon arc lamp is used to simulate sunlight, and an optical filtration system is used to simulate changes in the stratospheric ozone content.

Major Findings: A significant body of data has been generated through the use of the light source, Cary spectroradiometer, Robertson-Berger UV meters, and Wang computer. An important feature of the system is the ability to compare ozone and glass filter optical properties using one light source and one spectroradiometric detector system.

A summary of the first biological experiment has been submitted for publication (NCI Monograph 50). The results indicate, as expected, an increase in carcinogenic activity with decreasing filter thickness (simulating decreased ozone thickness). The relationship is internally consistent and of the same order of magnitude whether expressed in terms of risk (time to 50% incidence) or yield (time to average yield of one tumor).

Detailed information is available concerning the amount and distribution of light supplied to each experimental group. These data can not presently be used to generate a dose scale, however, since the appropriate weighting functions for the different spectral regions are not known. Instead, we intend to use the data from the second and third experiments, in which the dose is varied while the spectra remain constant, to relate a given response to a "dose-equivalent." In this way, we will back-calculate the "dose-equivalent" contribution of each spectral increment and indirectly estimate an action spectrum. A provisional interpretation of the first experiment is that short wavelength (<300nm) may be more effective than predicted by model action spectra currently in use. The second experiment is currently being analyzed, and the third will be completed in June 1978.

Significance to Biomedical Research and the Program of the Institute: This project will yield scientific data relevant to the broad field of cancer biology and in addition provide data of immediate concern to the National Cancer Institute (NCI), to NASA, and to other agencies concerned with inadvertent modifications of stratospheric ozone. Assessment of the contribution of various wavebands of ultraviolet light to changes in the skin will play an important part in the understanding of naturally-occurring light-induced alterations. One immediate benefit of the study is the availability of a sophisticated

spectrophotometric system for analysis of a variety of light sources. Several types of lamps have been analyzed (e.g., filament lamps, arc lamps, and fluorescent lamps) and the data made available to others involved in photobiologic research.

Proposed Course: The contract terminates June 30, 1978. An analysis of data will be submitted with the final report.

Date Contract Initiated: February 15, 1974

Current Annual Level: $234,469

TRACOR JITCO, INC. (NO1-CP-43350)

Title: Bioassay Prime Contract

Contractor's Project Director: Dr. Lorne A. Campbell

Project Officer (NCI): Dr. J. Fielding Douglas

Objectives: The purpose of the Bioassay Prime Contract is to provide scientific and management support to the Bioassay Operations Segment in the conduct of carcinogenesis bioassay testing of environmental chemicals. This support entails the following: (1) maintain responsibility for accurate and timely performance of bioassay subcontracts under the Prime; (2) evaluate and award new competitive and/or sole source subcontracts; (3) continue to coordinate and monitor the research conducted by the subcontractors: (4) propose and carry out scientific improvements and cost-saving/efficiency methods for the Program; (6) purchase chemicals and obtain chemical analysis information on compounds to be tested; (7) provide for data submission to the Carcinogenesis Bioassay Data System (CBDS) and assist in the preparation of final reports on the chemicals being tested; (8) establish and maintain a repository of residual bioassay materials and an archives of bioassay data; (9) evaluate, check, and improve the quality of pathology services on the Program, holding workshops as necessary to coordinate Program diagnoses; and (10) make a best effort in assuming other responsibilities as desired.

Major Findings: (1) Ten former NCI bioassay contracts were converted into Tracor Jitco subcontracts in previous fiscal years. These were Dow Chemical Company, Gulf South Research Institute, Hazleton Laboratories America, IIT Research Institute, Litton Bionetics, Inc., Mason Research Institute, Midwest Research Institute, Papanicolaou Cancer Research Institute, Southern Research Institute, and Stanford Research Institute. Since that time, six new subcontracts have been awarded. The new awards were made to Battelle Columbus Laboratories, Gulf South Research Institute, Industrial Bio-Test, Litton Bionetics, Inc., Mason Research Institute, and Southern Research Institute. Special-study subcontracts were awarded to Experimental Pathology Laboratories, Metcalf & Eddy, Papanicolaou Cancer Research Institute, the University of New Orleans, and Walden Division of Abcor. (2) Monitorship of the subcontracts was intensified. (3) A Management Plan and System for the Bioassay Operations has been continuously improved. (4) A list of prospective inhalation laboratories was developed

from a public announcement. The qualified bidders' list for oral studies was expanded. (5) A competitive Basic Ordering Agreement (BOA) for testing additional chemicals for future year's awards has been released. (6) The procurement of chemicals to be tested was continued. (7) Coordination of animal and chemical resources was continued. (8) Intensive activity on a series of reports on over 175 chemicals tested was carried out; other reports are in process now. (9) Actions were continued to maintain the flow of accurate and timely data into CBDS. (10) A repository and archives for bioassay materials has been established. (11) Pathology workshops have been held and interface between and among pathologists improved. (12) Safety improvements have been made in several areas of the Program; unannounced safety site visits are held routinely. A Safety and Health Plan has been developed and distributed. Subcontractor safety plans are being evaluated. (13) Cage systems have been investigated in depth and improvements obtained from two manufacturers. (14) Subcontractor equipment and facilities are being steadily upgraded across the board. (15) A revised Inhalation Bioassay Protocol has been developed and incorporated into an active contractual solicitation.

Significance to Biomedical Research and the Program of the Institute: One of the primary factors basic to the understanding and prevention of cancer is the identification of carcinogenic chemicals in the environment of man. If "environment" is defined to include not only the atmosphere, but also industry, transportation, clothing, housing, occupation, food, drugs, cosmetics, and any other entity with which man comes into contact, the number of suspect chemicals is very large. Thus, the number of chemicals which must be tested is large. Because of the nature of the bioassay, numerous animals must be utilized in testing each chemical over long-time periods. Extensive elements of data are produced which must be recorded accurately and interpreted expertly. This convergence of large-dimension factors results in high costs. To obtain the greatest value in knowledge with minimal expenditures of funds, it is essential that the Program be managed carefully to ensure good science, expert use of manpower and funds, and high quality. A principal goal of the Prime Contractor, therefore, is to achieve the objectives of the NCI Bioassay Operations Segment with high scientific credibility at reasonable costs.

Proposed Course: It is intended to continue ongoing bioassays of chemicals to completion, with subsequent reporting of results in the NCI Carcinogenesis Summary Reports Series. The testing of a substantial number of additional chemicals will be commenced according to a schedule arranged in terms of availability of funds, animals, chemicals, and qualified laboratories. Improvements in the bioassay procedures are planned with respect to sensitivity of animal models to carcinogens and identification of alternative animal models; development of time-dose-response procedures which can be used to supplement or replace screening procedures; employment of biochemical procedures to give a better understanding of metabolic pathways and detoxication mechanism involved during carcinogenesis tests; studying the design, environment of host factors that may affect the outcome of bioassay, e.g., feed, water, biometry; consideration of ways and means to implement cost-savings and efficiency by improvement in the design of laboratory facilities, streamlined methods of reporting pathology data, etc.

Date Contract Initiated: March 1, 1974

Current Annual Level: $12,379,849

D. TUMOR PATHOLOGY BRANCH

October 1, 1977 through September 30, 1978

"Plans, develops and conducts a program to (1) maintain a national tumor pathology reference center with emphasis on experimental tumors and their relationship to the human counterpart; (2) provide diagnostic pathology criteria, standardization, and monitoring for the Carcinogenesis Testing Program and related criteria for the development of experimental tumor models; (3) evaluate final test data for determination of carcinogencity; and (4) provide guidance, consultation and training in tumor pathology for both program and contract staff as well as consultation services to other national and international agencies on animal tumor pathology."

The primary goal of the Branch is to develop and maintain standards for pathology in carcinogenesis tests. To further this goal, during the past year the Branch developed a detailed review procedure for evaluating pathology in bioassays conducted under contract in the Carcinogenesis Testing Program. The procedure involves a review by members of the Pathology Working Group of quality assessment reports, pathology narratives and histological slides from these bioassays. The quality assessment procedures include verifying information in computer tables, tissue counts and proper diagnoses of lesions present on slides. The standardization of pathology procedures, nomenclature and diagnoses is continuing through these review procedures and pathology workshops.

A standard operating procedure guideline manual is being developed by the branch and contractors. In the past year, two workshops were held on the subjects of mouse liver tumors and pathology of the B6C3F1 mouse and F344 rat. The workshops provided a forum for discussing and understanding the lesions pathologists diagnose in rodent bioassays.

The Pathology Services Project of the National Center for Toxicological Research in Jefferson, Arkansas and the Tumor Pathology Branch cooperated in several studies and program functions. These included pathology workshops, standardization of terminology for mouse liver tumors and development of the Toxicology Data Management System, the new information system to be used by the Carcinogenesis Testing Program in fiscal year 1979.

Various bureaus of the Food and Drug Administration have received support and advice from members of the branch. Members have participated in the FD & C Red No. 40 Second Working Group, as an expert witness in hearings on FD & C Red No. 4 and as reviewers of a carcinogenesis study of a nitrofuran.

Intramural research was usually retrospective by design. Detailed reviews of the morphology of naturally-occurring and experimentally-induced hepatic neoplasms in mice provided valuable information which allowed the Branch to detail a classification scheme for these tumors. Morphology of induced tumors was frequently different from that of naturally-occurring tumors. Metastatic tumors were of several types. Morphologic studies of thyroid lesions induced by a hair dye component allowed us to postulate the possible mechanism of tumor induction. Quantative analysis of specific lesions was performed using

an image analysis computer. Further work in this field will be attempted. Quantitation of histologic lesions was attempted using "blind pathology analysis" and nonparametric statistical methods for bladder and colon carcinogenesis studies. The results were promising and future studies are planned. Utilization of suggested procedures for reducing the pathology workload in a bioassay revealed that 18% of the tissues did not have to be read by the pathologist. However, other disadvantages of these procedures were noted. A study comparing gross and histologic findings is under way.

Active recruiting for pathologists continued and 4 additional pathologists became staff members in the past year. However, the resignation of Dr. Dawn G. Goodman was a great loss to the Branch. With the addition of one pathologist, a program on pathology and carcinogenesis in aquatic animals was initiated. The goals of these studies are to provide and standardize comparative pathology information on tumors in aquatic animals and determine if aquatic animals can be used economically as bioassay screens for carcinogens, especially those found in aquatic environments. Cooperative studies with the Smithsonian Institution's Registry of Cancer in Lower Animals included field studies for tumors in aquatic animals and characterization of lesions in these species. Comparative analysis of lesions and tumors in aquatic animals and rodents should provide valuable information on the nature of these lesions.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER 04544 Z01 CP ~~04482~~-02 TP

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Carcinogenicity of 2,6-dimethylnitrosomorpholine (2,6-DMNM) in the Syrian Hamster

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. H. Levitt	Medical Pathologist	TP, NCI
OTHER:	M. S. Sporn	Chief, Lung Cancer Branch	LC, NCI
	M. Wenk	Research Scientist	Microbiological Associates

COOPERATING UNITS (if any)

None

LAB/BRANCH
Tumor Pathology Branch, Carcinogenesis Testing Program

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range purpose of this project to conduct tumorigenicity studies with <u>2,6-DMNM</u> in the Syrian golden <u>hamster</u> to confirm the effectiveness of this model to evaluate the role of <u>13-cis-retinoic acid</u> and related synthetic <u>vitamin A</u> derivatives on possible <u>inhibition</u> of hamster <u>pancreatic carcinogenesis</u>. The study will assess (1) the effectiveness of 2,6-DMNM in the Syrian hamster as a specific pancreatic carcinogen; (2) the number of induced neoplasms, if any, in organ systems other than the pancreas; (3) the effectiveness of the agent by both subcutaneous and intragastric routes of administration; and (4) histopathologic evaluation of the liver in terms of lethal or sublethal cellular injury (which must be minimized if a vitamin A analogue is to be successful in this model system).

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-~~04402~~ 04544-02-TP

Project Description

Objectives: To determine the effectiveness of 2,6-dimethylnitrosomorpholine (2,6-DMNM) as a specific pancreatic carcinogen in the Syrian golden hamster and to evaluate the role of 13-*cis*-retinoic acid and related synthetic vitamin A derivatives on the possible inhibition of pancreatic carcinogenesis.

Methods Employed: Three hundred forty outbred male Syrian golden hamsters, weighing 80-100 grams at the start, have received treatments according to the following:

Group[a] No.	No. of Doses	Each Dose	Route	Total Dose 2,6-DMNM	Dietary[b] Supplement
1	10(1x/week,10 weeks)	160mg/kg	IG	1600	Gelatin only
2	"	160mg/kg	IG	1600	CRA [c,d]
3	"	160mg/kg	SC	1600	Gelatin only
4	"	160mg/kg	SC	1600	CRA
5	"	80mg/kg	IG	800	Gelatin only
6	"	80mg/kg	IG	800	CRA [c,d]
7	"	80mg/kg	SC	800	Gelatin only
8	"	80mg/kg	SC	800	CRA
9	"	40mg/kg	IG	400	Gelatin only
10	"	40mg/kg	IG	400	CRA [c,d]
11	"	40mg/kg	SC	400	Gelatin only
12	"	40mg/kg	SC	400	CRA
13	"	0.2ml oil	IG	0	Gelatin only
14	"	0.2ml oil	IG	0	CRA
15	"	0.2ml oil	SC	0	Gelatin only
16	"	0.2ml oil	SC	0	CRA
17 (shelf control)					

[a]Carcinogen administrated in 0.2 ml olive oil. Animals necropsied at 15,25,40 weeks (6 animals each time). Hamsters were 8 weeks old male Syrian Golden at start of experiment.

[b]Basal ratio Purina Lab Chow in meal form

[c]CRA = 13-*cis*-retinoic acid (140mg/kg diet) in gelatin beadlets

[d]Dietary supplement replaced with basal rations 24 hours before DMNM administration until 24 hours after each dose.

Animals were examined by complete gross necropsy and routine light microscopy (to include all gross lesions, tissue masses or suspect tumors and regional lymph nodes, trachea/larynx, lungs/main stem bronchi, nasal cavity, liver, pancreas, spleen, and kidneys).

Major Findings: Preliminary evaluation of the histopathology indicates that the experimental animals developed a broad range of benign and malignant neoplasms involving the nasal cavity, lungs, liver and pancreas. The pancreatic neoplasms were similar to those previously reported with 2,6-DMNM and BHP (see below) and appear to arise from similar ductal components; viz., distal ducts and ductules. Preneoplastic change in the pancreatic ducts were present at 15 weeks, and neoplasms, benign and malignant, were seen in the 25 week period and beyond. Detailed evaluation of the groups to assess the effect of the retinoid is currently in progress. An additional finding was that animals dosed subcutaneously exhibited greater mortality than those dosed intra-gastrically with equivalent dosages.

Significance to Biomedical Research and the Program of the Institute: Pancreatic cancer is now the 4th leading cause of cancer mortality in the United States, with an incidence that is rising alarmingly. Since the introduction of the hamster model for pancreatic carcinogenesis by Pour et al. (Am. J. Pathol. 76: 349, 1974), demonstrating a high incidence of induced pancreatic neoplasms with N-nitroso-bis(2-hydroxypropyl)amine (BHP), considerable work on the metabolism of BHP and related symmetric di-alkylnitrosamines has suggested that N-nitroso-(2-hydroxypropyl) (2-oxopropyl) amine (HPOP) is a major metabolite (and possibly the proximate carcinogen) of the potent pancreatic carcinogen N-nitroso-bis(2-oxopropyl)amine (BOP), which is, of course, closely related to BHP. Moreover, HPOP and BHP are also putative metabolites of 2,6-DMNM in hamsters. Structurally, 2,6-DMNM and the cyclic acetal form of HPOP differ only in the presence of a hydroxy group at the 2-position in the latter compound. Preliminary investigations with 2,6-DMNM by Mohr et al. (J. Natl. Cancer Inst. 58: 429, 1977) have shown that when it is administered intragastrically to Syrian hamsters, over 70% of the animals developed pancreatic neoplasms, results also noteworthy for the significantly reduced incidence of tumors at other sites such as the respiratory tract and liver.

Proposed Course of the Project: Important studies confirming the inhibitory effect of the retinoids on tumorigenesis in several animal model systems including bladder, colon, esophagus, breast and lung have suggested a very important role for these vitamin A analogues as tumor inhibitors. The possibility of extending this work to the pancreas is the intent of this project.

Publications

None, as this project is in the evaluation stage.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04512-04 TP

PERIOD COVERED
October 1, 1977 through September 30, 1978

TITLE OF PROJECT (80 characters or less)

Morphogenesis of Pancreatic Adenocarcinoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. H. Levitt	Medical Pathologist	TP, NCI
OTHER:	C. C. Harris	Research Scientist	EXP, NCI
	R. A. Squire	Associate Professor	Johns Hopkins University, School of Medicine
	E. Kingsbury	Cell Biologist	Litton Bionetics
	M. Wenk	Research Scientist	Microbiological Associates
	S. Springer	Biologist	Gulf South Research Institute

COOPERATING UNITS (if any)

None

LAB/BRANCH
Tumor Pathology Branch, Carcinogenesis Testing Program

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range purpose of this project to delineate the pathogenesis and morphogenesis of pancreatic adenocarcinoma in the Syrian golden hamster, induced by N-nitroso-bis(2-hydroxypropyl)amine (BHP), a model introduced by Pour et al. (Am. J. Pathol. 76: 349, 1974). The topics of present interest are (1) the histologic appearance of the induced pancreatic neoplasms by high-resolution light (1 μm sections) and transmission electron microscopy; (2) characterization of the cell(s) of origin of the induced neoplasms and their comparison to human pancreatic cancer cells; (3) the extension of the model to include larger experimental animals, such as the guinea pig, to broaden the scope of experimental pancreatic carcinogenesis research; (4) distribution of the carcinogen in the animals, employing radiolabelled BHP, to determine the localization of the putative proximate carcinogen in the target organ(s); and (5) identification of preneoplastic markers in pancreatic carcinogenesis and their applicability as possible diagnostic tools for the early detection of malignant process.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04512-04-TP

Project Description

Objectives: To characterize the pathogenesis and morphogenesis of pancreatic carcinoma induced by N-nitroso-bis(2-hydroxypropyl)amine (BHP; DIPN; DHPN) through the use (1) high resolution (1 μm sections) light microscopy, (2) transmission electron microscopy, and (3) quantitative microscopic autoradiography.

Methods Employed: Fifty-two test and 13 control outbred male Syrian golden hamsters, weighing 80-100 grams at the start, received BHP (250 mg/kg body wt.; 1250 mg/M^2) sc, suspended in olive oil, once weekly. They were killed in groups of 4 test and 1 control at 1 hour, 1, 2, 5, 10, 12, 15, 17, 20, 22, 26 and 31 weeks one hour after receiving an ip injection of ^{3}H-TdR (3.0 μCi/gm body wt.; specific activity 6.7 Ci/mM). Sections of pancreas were fixed and plastic embedded for high resolution (1 μm sections) light and transmission electron microscopy, and developed for quantitative light autoradiography. A second parallel study was conducted wherein distilled H_2O was substituted for the olive oil as the vehicle for the carcinogen. A third experiment sought to reproduce the findings of Pour et al. (Am. J. Pathol. 76: 349-358, 1974), who obtained a high incidence of pancreatic neoplasms in a lifetime study with outbred Syrian golden hamsters following weekly sc applications of BHP, suspended in olive oil, with a tumor latency as short as 15 weeks. A fourth parallel experiment, employing 40 test and 20 control animals per group was conducted at three dose levels (250, 125 and 25 mg/kg body wt.) of BHP dissolved in distilled H_2O, to determine the effect of a hydrophilic solvent on tumorigenesis. A fifth experiment will seek to examine the uptake and localization of carcinogen in the pancreas and other tissues by means of injection of ^{14}C-BHP or ^{3}H-BHP (at the α-position) and serial sacrifice at 5 time points of a group of 25 animals over a 72-hour period. A sixth experiment was conducted to determine the pancreatic carcinogenic potential of BHP in inbred (strain 13) guinea pigs, wherein groups of 10 male and 10 female weanling animals weighing 250-300 grams at the start received weekly sc injections of BHP in H_2O at 5 dose levels (1000, 500, 250, 125 and 63 mg/kg body wt.), with a control group of 20 animals, for a 90-day period, to establish a maximum tolerated dose for the guinea pig in anticipation of a chronic study similar in design to the hamster lifetime studies.

Major Findings: The results of the morphogenesis studies in hamsters were consistent with the hypothesis that the induced pancreatic neoplasms arise from duct epithelium (most probably ductular cells), progressing from duct hyperplasia to ductal cystadenomas and papillary cystadenomas, and finally to intraductal and invasive ductal carcinomas. No acinar cell neoplasms were observed. The tumors were usually multicentric and had a predilection for the "body" and "tail" regions of the hamster pancreatic lobes. Metastases were common to local and regional lymph nodes, and occasionally to liver and lungs. Invasion of contiguous structures such as stomach, intestinal wall and spleen were commonly noted. Electron microscopy showed early non-specific damage to acinar as well as duct cells, but the acinar cells underwent regeneration and/or degeneration while the duct cells underwent neoplastic

transformation. There was some evidence to suggest that a consistent and distinctive appearance of the nuclear chromatin pattern in the duct cells heralded the appearance of malignant transformation. Other histologic patterns observed with the malignant pancreatic neoplasms included cribiform proliferation, cellular anaplasia, periductal fibrosis and chronic inflammation and unusual and bizarre nuclear and nucleolar forms.

Autoradiographic data provided a good correlation between the biology and histogenesis in the hamster model system. Although a gradual increase in labelling of duct cells with time was noted, there was nonetheless a dramatic multifold increase at 22 weeks (BHP in olive oil) and 10 weeks (BHP in H_2O), corresponding precisely to the times when overt neoplasms (adenomas and carcinomas) became histologically apparent. These data were significant ($p < 0.05$) using the standard parametric two-tailed _t_-test as well as a non-parametric randomization test.

The results of the lifetime studies compared favorably with those of Pour et al. (_op. cit._), with at least a 73% incidence of pancreatic ductal neoplasms in the (25 mg/kg. body wt.) lowest dose group and a 94% incidence in the (250 mg/kg body wt.) highest dose group (both H_2O vehicle). The incidence was 100% in the (250 mg/kg body wt.) BHP-olive oil group. An unexpected finding was a very high incidence of hepatic angiosarcomas (range 46-56%). Also encountered were significant numbers of nasal cavity (range 30-65%), pulmonary (range 30-76%), tracheal (range 7-22%), gastric (range 2-17%), colonic (range 2-5%), and other neoplasms including those of renal and hematopoietic origin. Another interesting finding was that of megalocytosis in non-neoplastic urothelial, renal and hepatic epithelial cells, which may be direct result of the carcinogen (abnormal cell division). There was no electron microscopic evidence of a viral etiology to the megalocytosis.

The effect of substituting H_2O for olive oil as the vehicle for the carcinogen was to decrease the average latency period for pancreatic tumor development from 19 to 16 experimental weeks in the comparable groups (250 mg/kg body wt.). With the H_2O vehicle, the effect of lowering the dose from 250 to 125 mg/kg body wt. resulted in increasing average survival from 23 to 34 weeks, produced about the same yield pancreatic tumors (94% and 100%, respectively), but dramatically increased the yield of tumors in other organs. At the 25 mg/kg dose level 75% of the animals developed pancreatic neoplasms.

The results of the dose-ranging study with BHP in the strain 13 guinea pigs revealed that all doses of BHP except the lowest, 62.5 mg/kg body wt., resulted in greater than a 10% body weight suppression in 13 weeks. Even at the lowest dose, weight loss was approximately 8-10%. Mortality was unaffected in those receiving the two lowest doses, but clinical signs of toxicity, weight loss, lethargy and rough hair coat were evident in all animals as well as microscopic evidence of liver pathology, including hemorrhageic necrosis, micronodular cirrhosis, hepatocellular carcinoma and angiosarcoma. No evidence of pancreatic pathology, except for focal and lobular acinar cell degeneration was evident, suggesting the refractive nature of the strain 13 guinea pigs to BHP-induced pancreatic carcinogenesis.

Accordingly, projected protocols for a large scale chronic dosing study were not implemented, and a small pilot study, wherein groups of 5 male and 5 female animals continued to be dosed, was begun. The animals employed were from the original dose-ranging study, receiving 250, 125, 62.5 or 0 mg/kg/week BHP, for life. Although there were dose-related survival and weight decrements, the animals remained refractive to induced pancreatic carcinogenesis to termination at 46 experimental weeks. Pulmonary and hepatic neoplasms did occur in closed groups only, indicating carcinogenesis, albeit not pancreatic, was induced.

Significance to Biomedical Research and the Program of the Institute: Pancreatic cancer is now the 4th leading cause of cancer mortality in the United States, with an incidence that is rising alarmingly. Until the emergence of the hamster-BHP model in late 1974, no adequate animal model was available for the induction and study of pancreatic carcinoma. The project has reaffirmed the significance of this model, delineated the morphogenesis and provided a baseline for further studies by the biomedical research community in an effort to determine the etiology and search for early methods of detection and prevention of the human disease.

Proposed Course of the Project: Detailed analysis of the guinea pig study indicated that these animals were refractory to BHP-induced pancreatic carcinogenesis. This observation in the guinea pig as compared to the hamster could feasibly serve as an experimental system in which evaluation of factors involved in BHP-induced pancreatic carcinogenesis could be further studied with respect to inter-species differences in metabolism, etc. Plans are currently being formulated to examine the efficacy of such a study. The carcinogen uptake and localization experiment in the hamsters will not be implemented since this is being done at another institute. Further experiments with the BHP-hamster model are in the planning stages, including a proposed study of L-asparagine synthetase as a biochemical marker for malignant pancreatic neoplasia in the Syrian golden hamster dosed with BHP and/or related carcinogens.

PUBLICATIONS

Levitt, M., Harris, C., Squire, R., Springer, S., Wenk, M., Mollelo, C., Thomas, D., Kingsbury, E., and Newkirk, C.: Experimental pancreatic carcinogenesis. I. Morphogenesis of pancreatic adenocarcinomas in the Syrian golden hamster induced by N-nitroso-bis(2-hydroxypropyl)amine. Am. J. Pathol., 88: 5-28, 1977.

Levitt, M., Harris, C., Squire, R., Wenk, M., Mollelo, C., and Springer, S.: Experimental pancreatic carcinogenesis. II. Lifetime carcinogenesis studies in the Syrian golden hamster with N-nitroso-bis(2-hydroxypropyl)amine. J. Natl. Cancer Inst. 60: 701-705, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04548-05-TP

PERIOD COVERED
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Registry of Experimental Cancers

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Harold L. Stewart	NIH Scientist Emeritus	TP NCI
OTHER:	Thelma B. Dunn	Consultant	TP NCI
	Margaret K. Deringer (Margaret D. Miller)	Biologist	TP NCI
	Bernard Sass	Veterinary Medical Officer	TP NCI
	Cornelia Hoch-Ligeti	Guest Worker	

COOPERATING UNITS (if any)

NONE

LAB/BRANCH
Tumor Pathology Branch, Carcinogenesis Testing Program

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	3.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1. The objectives of the Registry of Experimental Cancers are 1) the storage and retrieval of pathologic material and data on cancers and other lesions of laboratory animals (primarily rodents) and 2) the use of such information for research and educational purposes.
2. The Registry has acquired a total of 1335 (366 since the 1977 report) single or group accessions from investigators outside of NCI. Approximately 38,000 (10,000 since the 1977 report) records have been prepared for coding and coded.
3. Forty-four investigators have come to the Registry for consultations on single or multiple visits.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04548-06-TP

Project Description

Objectives: 1) The storage and retrieval of pathologic material and data on cancers and other lesions of laboratory animals (primarily rodents), and 2) the use of such for research and educational purposes.

Methods Employed: The methods employed in the work of the Registry involve the selection of protocols, pathologic material, including histologic slides, paraffin blocks, and gross specimens; and illustrations in the form of lantern slides, gross photographs, and photomicrographs in black and white and in color. The work of the Registry also includes the collection of records of experiments, reprints and references on this material. The Registry of Experimental Cancers possesses a large collection of spontaneous and induced cancers and other lesions. The pertinent information on the collection is being indexed. Many of the data have been prepared for and entered into the computer. The Registry accesses material from investigators at NCI, other institutes of NIH, other governmental agencies, industrial laboratories, and universities here and abroad. A total of 1335 single or group accessions from investigators outside of NIH have been processed since 1971. The Registry is preparing Study Sets of slides, with explanatory material, relating to particular areas of cancer in rodents.

The Registry has Study Sets of slides on "Hematopoietic and Lymphoreticular Neoplasms", "Induced and Spontaneous Tumors of Small Intestine and Peritoneum in Mice", "Tumors and Nonneoplastic Proliferative Lesions of the Lungs of Mice", and "Induced Tumors of the Liver in Rats", which are loaned with descriptive material, to investigators who request them. Seventeen loans up to two months have been made this year.

Investigators come to the Registry for consultation. There have been single or multiple consultations with 44 individuals since April 1977.

Major Findings: The functions (outlined in objectives) of the Registry in the wider field of cancer research and more particularly in the Carcinogenesis Testing Program are such that there will be no major findings to report.

Significance to Biomedical Research and the Program of the Institute: The availability of the wealth of material possessed by the Registry advances the knowledge of spontaneous and induced disease processes in animals.

The existence of the Registry will contribute to the standardization of nomenclature of cancers and other lesions in laboratory rodents. Slides and protocols from the Registry are used to illustrate and describe lesions discussed at the weekly slide conferences.

The members of the Registry serve as consultants in the monitoring of pathology from laboratories under the Carcinogenesis Testing Program.

The NAS, NRC, ILAR Subcommittee on Rat Liver Tumors Committee on Histologic Classification of Laboratory Animal Tumors has held all of its meetings, 9 to date, at the Registry. The subcommittee is using the Registry's Study Set of "Induced Tumors of the Liver in Rats" as a basis for the histologic typing of liver tumors in this species. The histologic classification and typing of tumors in laboratory animals is calculated to promote uniformity of diagnoses from one laboratory to another in this country. This should be of inestimable value to the Carcinogenesis Testing Program.

The Director-General of the World Health Organization designated the Registry of Experimental Cancers as the WHO Collaborating Centre for Reference on Tumours of Laboratory Animals on 26 October 1976. This is the only such registry in the world to be so designated by the WHO. This will expand communications between scientists of this country and those of other countries, now numbering 153, which are members of WHO.

Proposed Course of the Project: The Registry will continue and expand all of its activities (already set forth in this report).

Publications

Stewart, H. L.: Enigmas of cancer in relation to neoplasms of aquatic animals. Ann N Y Acad Sci. 298:305-315, 1977.

Stewart, H. L.: Fathollah Keshvar Mostofi A Profile. Internat. Pathol. A News Bulletin 18:No.4, 1977.

D. TUMOR PATHOLOGY BRANCH
CONTRACT NARRATIVES
October 1, 1977 through September 30, 1978

AMERICAN HEALTH FOUNDATION (N01-CP-75940)

Title: Long Term Studies of Prevention of Epithelial Cancer by Retinoids

Contractor's Project Directors: Dr. John H. Weisburger
Dr. Jerald Silverman

Project Officers (NCI): Dr. Carl E. Smith
Dr. Michael Sporn
Dr. Jerrold M. Ward

Objectives: The objective of this program is to assess the effects of retinoids on epithelial tissue, specifically that found in the colon using the direct acting carcinogen *N*-methyl-*N*-nitrosourea and the indirect acting carcinogen 1,2, dimethylhydrazine to induce tumors. Since it is of major importance to be able to detect early neoplastic lesions of the colon, endoscopy on the rats will be used in this study to detect early colonic lesions. The long term objective will be a dose response study to determine what level of dietary retinoid is needed to prevent neoplastic changes.

Major Findings: This is a new contract and experiments have been under way since January, 1978. There are twenty-five groups of Fischer F344 rats involving a total of 667 animals given the colon carcinogens 1,2, dimethylhydrazine subcutaneously, or *N*-methyl-*N*-nitrosourea intrarectally. There are control animals as well as rats given five distinct retinoid compounds.

Significance to Biomedical Research and the Program of the Institute: Supported by another Contract (N01-CP-33208) American Health Foundation has pioneered in the development of animal models for colon cancer research. Several of these models are currently being applied to induce early colon tumors and to study whether the course of this induction process can be modified by retinoids. Collateral data will be collected to acquire information on the mechanism whereby any inhibition occurs. Colon cancer is the cancer with the highest incidence in the United States in particular, in the Western world in general. Any valid information permitting a reduction of the impact of this disease will be a valuable asset.

Proposed Course: (1) To continue the experiments designed to investigate the effect of retinoids in colon cancer in animal models. (2) To acquire data bearing on the mechanism of this induction process.

Date Contract Initiated: September 30, 1977

Current Annual Level: $125,000

CALIFORNIA, UNIVERSITY OF (at Davis) (NO1-CP-65845)

Title: Biology of Neoplastic Liver Lesions in Mice

Contractor's Project Director: Dr. Boris Ruebner

Project Officer (NCI): Dr. Jerrold M. Ward

Objectives: To investigate the biological characteristics of liver tumors induced in mice. To achieve this goal, enzyme histochemistry, tumor transplantation and morphologic studies will be employed. Biological characteristics of tumors in the livers of C57BL/6 and C3H (and their hybrids) mice produced by compounds (i.e. dieldrin) which have a negative or marginal effect at other organ sites and in other species will be compared to tumors produced by a compound (DEN) which is an unequivocal carcinogen. Early liver lesions will be defined and their relationship to the development of overt hepatocellular carcinoma will be characterized.

Major Findings: Weanling male C57BL/6 mice, a strain with a low rate of spontaneous incidence of hepatic neoplasms, weanling male C3H mice, a strain with a high rate, and male C57/C3H hybrids were employed in the studies herein. Diethylnitrosamine (12 mg/kg) was given intraperitoneally; dieldrin was fed continuously at 10 parts per million; and appropriate controls were used.

Three months after initiation of this experiment, monthly sequential examinations began. Additionally, at 12 months of age, a time for which approximately 50% of the dieldrin-fed mice had signs of gross hepatic nodules, animals from each group were removed for serial observations by diagnostic laparotomy. Samples from each hepatic nodule and of non-nodular liver were taken for light and electron microscopy. Selected enzyme histochemistry was performed. Laparotomy was performed under ether anesthesia using a sterile rodent surgical kit. Visible nodules were needle biopsied and analyzed as above. Moreover, gross nodules were transplanted into congenitally athymic (nude) mice.

The only significant pathologic changes were noted in the livers. For the first 8 months of the experiment, no focal changes could be seen grossly in any of the three groups of animals. Tiny pale capsular lesions and larger hepatic nodules were first seen in the DEN and dieldrin C3H animals after 9 months.

The livers of the control animals and of the DEN animals showed no diffuse changes. The dieldrin animals, however, showed marked swelling of hepatocytes in central zones associated with some nuclear atypia.

Scanty scattered single clear cells or small foci of altered hepatocytes with clear cytoplasm appears for the first time in the DEN animals 8 months after administration. The larger foci of altered cells were generally heterogeneous and consisted of a mixture of clear and basophilic cells, usually found at the periphery of the focus.

The nodules found in the DEN and dieldrin groups were generally heterogeneous and contained clear, basophilic and intermediate cells in a random pattern. Histochemically, most of the cells in the nodules were deficient in all the enzymes investigated. Two animals in the dieldrin group and also two in the DEN group had nodules which were considered to be hepatocarcinomas. No metastases were detected in the lungs of these animals. The "benign" appearing nodules were not transplantable into nude mice; one "malignant" nodule was transplantable.

The C57BL/6 animals showed nodules at 13 months (four months later than the C3H). No definite lesions have been demonstrated, so far, in the C3H/C57 hybrids. However, these animals are only approximately 8 months old at the time of this writing.

Ultrastructurally, no significant differences were detected between the nodules of the dieldrin and nitrosamine animals. The clear cells in the nodules differed from the hepatocytes of controls in that glycogen areas contained conspicuous fat droplets. The basophilic and intermediate cells resembled the clear hepatocytes in their general structure. The principal difference was an increase in rough endoplasmic reticulum.

Serial observations of C3H mice, undergoing diagnostic laparotomy has revealed a continuously increasing size of individual hepatic nodules. Because of the size of the well exposed surgical field, these nodules have been photographed to permit sequential analysis. Of particular interest is that individual nodules of all groups continue to grow, even in animals who have previously discontinued dieldrin. Thus, preliminary evidence suggests, in the disease and experimental design herein, no signs of nodule regression. This increment was apparent, and of the same magnitude, whether dieldrin was continuously fed or had been discontinued.

Significance to Biomedical Research and the Program of the Institute: Since the mouse is one of the most commonly used animals in carcinogenesis research, including long term bioassay, an understanding of the biology of neoplastic liver lesions in this species is important. Comparison of biologic properties of neoplastic liver lesions should lead to the determination of whether unifying criteria for classification exist.

Proposed Course: Serial monitoring, transplantation, and laparotomies of all groups of mice are in progress. Additionally, analysis of hepatic tissue and sera for glucose-6-phosphatase, succinic dehydrogenase, ATP-ase, proline hydroxylase and alpha-fetoprotein are in progress for the C3H group. The common denominator and area for particular interest in these studies are the observations on "pre-malignant" lesions and apparent irreversibility of the dieldrin-induced lesions. Emphasis on the latter continues.

Date Contract Initiated: September 30, 1976

Current Annual Level: $180,000

CHARLES RIVER BREEDING LABORATORIES, INC. (NO1-CP-55651)

Title: Establishment of a Gnotobiote Originated Rodent Production Colony

Contractor's Project Directors: Dr. Albert P. Otis

Project Officer (NCI): Dr. Thomas Cameron

Objectives: To establish breeding colonies to produce Fischer inbred 344 rats at a level of 2,000 per month and B6C3F1 hybrid mice at the projected level of 4,000 per month. Breeders for the "barrier" breeding colonies will be supplied by pedigreed expansion colonies of association flora status animals which will be maintained in isolator systems. The major objective of this program is to supply the Government with animals of defined quality in sufficient numbers to maintain the level of the Carcinogenesis Testing Program.

Major Findings: Because of the exactness required to develop sufficient pedigreed isolator stock for this Program in addition to those germfree animals supplied by the Government, the Contractor had to caesarian derive in germfree isolators additional pedigreed animals to increase its capability to meet the increased requirements by the Government. In addition to the initial breeding stock necessary to support this effort, a recycling program will take place on a routine basis in order that there not be a genetic shift from the animals produced by the Contractor and those maintained at NCI. The current goal to support the production requirements noted above would require associated pedigreed isolator foundation stock bred on a one to one basis of 60 pair of F344 inbred rats, 240 pair of C3H/He and 420 pair of C57BL/6. When the quantities required in the foundation colonies are reached, 500 pairs of F344's should be sufficient to produce the 2,000 rats required monthly. The C57BL/6 females x the C3H/He males at a paired level of 1,200 should produce 4,000 B6C3F1 hybrids monthly.

Significance to Biomedical Research and the Program of the Institute: Producing animals from isolator derived colonies should enable researchers to utilize animals of uniform quality with little or no variability. The major reason for enabling this to be accomplished is the maintenance of a breeding stock for the production colonies in germfree isolator systems. It will also provide assurance that should difficulty arise in the production colonies, defined animals can be made immediately available for production replacement.

Proposed Course: The program as outlined in the contract is progressing within the limitations of the associated germfree pedigreed foundation stock. On February 23, 1978, the Contractor began setting up a new production colony from isolators at the Portage, Michigan facility. This was to be completed by July 1, 1978. The pedigreed isolator colony will remain at the Wilmington, Massachusetts location.

Date Contract Initiated: October 1, 1974

Current Annual Level: $219,408

EXPERIMENTAL PATHOLOGY LABORATORIES, INC. (N01-CP-65731)

Title: Animal Pathology Support

Contractor's Project Director: Dr. William M. Busey

Project Officer (NCI): Dr. Jerrold M. Ward

Objectives: (1) To provide consultation services to the Chief of the Tumor Pathology Branch; (2) to perform designated histopathological evaluations of animal tissues from intramural and extramural programs within the Carcinogenesis Research and Testing Programs; and (3) to provide training in histologic techniques for contractor personnel in the Carcinogenesis Testing Program.

Major Findings: During the past year, histopathologic evaluation of tissues from rats and mice receiving Sodium Diethyldithiocarbamate, Ledate, and Ortho-Toluidine Hydrochloride was conducted. Histopathologic evaluation of rats receiving Amanthrene was also conducted. Specific findings of these histopathologic evaluations were submitted to the Pathology Working Group, Tumor Pathology Branch with the pathology narrative and will be incorporated into the NCI technical report for each compound.

The histopathology for the rats on the Sodium Diethyldithiocarbamate study was performed according to procedures developed by Dr. Thomas R. Fears and Dr. J. F. Douglas of the National Cancer Institute. These suggested procedures were intended to reduce the pathology workload in the Carcinogenesis Testing Program. The results of this practical evaluation of these procedures confirmed that they do save pathology time. Positive and negative aspects discovered during the practical evaluation were submitted to and are currently being reviewed by the National Cancer Institute.

The pathologists at EPL participated in the activities of the Pathology Working Group. The pathologists reviewed slides and pathology narratives submitted to NCI. EPL's pathologists also participated along with other members of the Pathology Working Group in initial discussions pertaining to quality assurance of the pathology for the Testing Program. EPL has helped to draft histology procedures to be incorporated into an overall quality assurance program.

Pathologists at EPL participated in a Mouse Liver Tumor Workshop, held at the National Center for Toxicological Research, November 3-4, 1977.

The histology laboratory at EPL prepared a duplicate set of slides for the mice receiving Ledate. These slides were sent to the National Center for

Toxicological Research in November 1977. The histology laboratory also prepared sections of stomach and bladder from several bioassay studies performed at Stanford Research Institute. These sections of stomach and bladder were reviewed by Dr. Ward, Tumor Pathology Branch.

During the past year, Mr. Dalton Tidwell, BioMed Systems, Inc., completed documentation of changes which were made to the original SNOP during its modification into the NCI Bioassay "MINI-SNOP" for the nontumor pathology sections (1000-7000 series). Work is currently proceeding on the tumor pathology sections (8000 and 9000 series).

Significance to Biomedical Research and the Program of the Institute: This Animal Pathology Support contract provides a great deal of flexibility to the Carcinogenesis Testing Program contractors and to the Chief of the Tumor Pathology Branch. The personnel and facilities of Experimental Pathology Laboratories are available to the Program contractors and to various intramural investigators in the Carcinogenesis Programs. The pathology services provided by EPL allow for the timely completion of the histopathologic evaluation of compounds under investigation in the Carcinogenesis Testing Program. Intramural investigators within the Carcinogenesis Research Program may use the facilities and personnel of EPL to assist them in the conduction of their research programs. The pathologists at EPL are available for consultation, and the histology laboratory of EPL is available for the processing of animal tissues for histologic examination. Similarly, the histology laboratory at EPL is available for the training of intramural and extramural histology technicians.

Proposed Course: To continue this pathology support service to the Carcinogenesis Programs for as long as it is required.

Date Contract Initiated: April 20, 1976

Current Annual Level: $244,903

HARLAN INDUSTRIES, INC. (NO1-CP-65737)

Title: Establishment of a Rodent Production Colony

Contractor's Project Director: Mr. Hal P. Harlan

Project Officer (NCI): Mr. Clarence Reeder

Objectives: To develop a rodent production colony housed under strict barrier conditions in the shortest possible time, and to attain an issuable weanling level of 2,000 Fischer Inbred 344 rats and 4,000 B6C3F1 hybrid mice of equal sex on a monthly basis. As of January 1, 1978 the contract was increased to attain an issuable weanling level of 4,000 Fischer Inbred 344 rats and 8,000 B6C3F1 hybrid mice as soon as possible. Desired levels were projected to be completed by mid 1978.

Major Findings: Original production of inbred F344 rats and B6C3F1 hybrid mice have been established at desired levels.

Significance to Biomedical Research and the Program of the Institute: The significance of this contract is that it will provide a constant and desirable source of animals to the various NCI contractors, enabling them to continue their programs which utilize the strains being produced. It will also insure the use of a standard biological mode to those investigators performing related experiments.

Date Contract Initiated: June 24, 1976

Current Annual Level: $609,000

IIT RESEARCH INSTITUTE (N01-CP-75939)

Title: Long Term Studies of Prevention of Epithelial Cancer by Retinoids

Contractor's Project Director: Dr. Richard C. Moon

Project Officers (NCI): Dr. Carl E. Smith
Dr. Michael B. Sporn
Dr. Sherman F. Stinson

Objectives: The major goal of this project is to assess the chemopreventive activity of retinoids (natural and synthetic analogues of vitamin A) in long term studies conducted in rodent models for breast cancer.

Major Findings: Previous work has indicated that retinyl acetate suppresses the appearance of mammary adenocarcinomas induced in Sprague-Dawley female rats by 1-methyl-1-nitrosourea (MNU) or 7,12 dimethylbenz(a)anthracene (DMBA). In ongoing long term carcinogenesis studies, retinyl methyl ether, retinyl butyl ether and axerophthene have suppressed the appearance of palpable mammary tumors induced in Sprague-Dawley female rats by MNU. However, since these studies are still in progress, confirmation of the chemopreventive activity of these retinoids against mammary carcinogenesis must await the histopathological evaluation of the palpable tumors subsequent to the termination of the studies.

Significance to Biomedical Research and the Program of the Institute: Studies performed under this contract, although still in progress, indicate that efforts to synthesize organotrophic retinoids with increased chemopreventive activity and diminished toxicity in comparison to previously tested compounds, are meeting with success. The data obtained from long term evaluation of retinoids will hopefully lead not only to the establishment of the concept of cancer chemoprevention, but will also provide evidence for the use of retinoids in suppressing progression of early neoplastic lesions in women who are at high risk for breast cancer.

roposed Course: Newly synthesized retinoids will be evaluated for chemopreventive activity in rodent models for breast cancer.

Date Contract Initiated: September 30, 1977

Current Annual Level: $123,000

JOHNS HOPKINS UNIVERSITY (N01-CP-65768)

Title: Animal Pathology Support

Contractor's Project Directors: Dr. John D. Strandberg
Dr. Linda C. Cork

Project Officer (NCI): Dr. Jerrold M. Ward

Objectives: This project provides pathology support to the intramural and extramural components of the Carcinogenesis Research and Testing Programs, utilizing histochemistry, light and electron microscopy. The contract pathologists participate in advisory groups and workshops for the Carcinogenesis Testing Program and related activities. They study experimentally-produced lesions with a view to ascertaining the progression of the lesions and identification of preneoplastic states. Lesions are also studied from a comparative viewpoint to document morphologic and functional similarities to spontaneous tumors of man and animals. Finally, this program supports training for veterinarians and physicians in the field of tumor and comparative pathology.

Major Findings: The pathologists regularly participate in informal working groups which critically evaluate the pathology in the Testing Program. It is through the efforts of these groups that the majority of the backlog has been eliminated in this program over the past months. Special morphologic and histochemical studies have been conducted on hepatocellular tumors of rodents, a perithelial sarcoma of mice, granular cell neoplasms of the peritoneal cavity of mice, and on crystalline inclusions in the livers of mice fed Trichlorvinphos. Both principal pathologists are participating in a workshop on murine neoplasms to be held for pathologists in the Testing Program. Dr. Strandberg will discuss and present tumors of the female reproductive system and Dr. Cork will cover central nervous system tumors. A research associate supported by this contract, Dr. David Brownstein, completed his training in comparative pathology in November of 1977 and successfully completed the Board examination for certification by the American College of Veterinary Pathologists.

Significance to Biomedical Research and the Program of the Institute: These activities have helped to upgrade the level of pathology in the Carcinogenesis Testing Program and have also been instrumental in standardizing terminology throughout a variety of laboratories. The results of important carcinogenesis testing assays have been made available and have allowed appropriate

responses on the part of regulatory agencies. The studies in pathogenesis and histogenesis of a variety of neoplasms have extended the field of knowledge in experimental carcinogenesis. A trained veterinary pathologist with knowledge of laboratory animal and tumor pathology has been added to the group of individuals qualified to participate in carcinogenesis programs. Additional individuals are being trained at the present time.

Proposed Course: Service, investigative and training aspects will continue. A study following the development of tumors in irradiated and non-irradiated *Mastomys sp.* will be completed. A follow-up study designed to ascertain the prognosis of spontaneous skin, soft tissue, and mammary tumors in dogs will also be completed. The training of tumor and comparative pathologists will continue with the recent appointment of a new resident.

Date Contract Initiated: March 22, 1976

Current Annual Level: $164,117

MARYLAND, UNIVERSITY OF (NO1-CP-65752)

Title: The Biology of Neoplastic Liver Lesions in Mice

Contractor's Project Directors: Dr. David E. Hinton
Dr. Benjamin F. Trump

Project Officer (NCI): Dr. Morton H. Levitt

Objectives: This project seeks to determine the biological characteristics of liver tumors induced in mice. To achieve this goal, *in vitro* cell and organ culture, enzyme histochemistry, tumor transplantation, intermediate energy metabolite, and morphologic assays will be used. Biological characteristics of tumors in the livers of BALB/c mice produced by compounds (i.e., Dieldrin, DDT) which have a negative or marginal effect at other organ sites and in other species will be compared to tumors produced by a carcinogen (Safrole) which is known to cause unequivocal liver cancer in the mouse and in the rat. Early liver lesions will be defined and their relationship to the development of overt hepatocellular carcinoma will be achieved.

Major Findings: BALB/c mice have been fed diets containing Safrole, Dieldrin and DDT and studies have been completed through 36 weeks. Similar tissue alterations have been demonstrated in all 3 groups through 16 weeks of exposure. The livers contained centrolobular foci composed of hypertrophic hepatocytes with varying degrees of lipid accumulation, especially prominent in the Safrole mice. By electron microscopy the hypertrophic hepatocytes displayed smooth endoplasmic reticulum (SER) proliferation. The Dieldrin and DDT exposed mice appeared the same at 24 and 36 weeks as at the earlier time periods. Additional findings in the Safrole mice at 24 weeks included extensive oval cell proliferation and midzonal to centrolobular lipid accumulation. Hepatocellular changes included the appearance of foci of small,

basophilic cells and clear cells. At 36 weeks Safrole mice displayed gross nodules 0.5 to 3mm. Histologically the nodules were composed of basophilic hyalinized and occasional fat cells. The surrounding parenchyma was compressed. The nodules occurred in 40% of the mice.

Significance to Biomedical Research and the Program of the Institute: Since the mouse is one of the most commonly used animals in carcinogenesis research, including long term bioassay, an understanding of the biology of neoplastic liver lesions in this species is important. Comparison of biologic properties of neoplastic liver lesions should lead to the determination of whether unifying criteria for classification exist.

Proposed Course: Long term dietary exposure of male BALB/c mice to DDT, Dieldrin, and Safrole will be done to produce tumors for biologic study and comparison. Serial exposures will be carried out to determine stages in neoplastic development. Finally, studies will be done to determine which of the lesions produced can be reversed by cessation of exposure.

Date Contract Initiated: September 30, 1976

Current Annual Level: $66,700

MICROBIOLOGICAL ASSOCIATES, INC. (N01-CP-02199)

Title: Laboratory Service for Support in Carcinogenesis Bioassay and Related Activities

Contract Narrative is reported under the Biology Operational Unit, Carcinogenesis Research Program.

MIDDLESEX HOSPITAL MEDICAL SCHOOL (N01-CP-75938)

Title: Long Term Studies of Prevention of Epithelial Cancer by Retinoids

Contractor's Project Director: Dr. R. Marian Hicks

Project Officers (NCI): Dr. Carl E. Smith
Dr. Michael B. Sporn
Dr. Morton H. Levitt

Objectives: The objective of this program is to determine the relative effectiveness of different retinoids in preventing and/or reversing neoplastic growth of the epithelium lining the urinary bladder of carcinogen-treated rats. This will be assessed both by long term studies in which the life-time response of carcinogen-treated animals to retinoids is examined, and also by careful histological and electron microscopical assessment of the subcellular

changes occurring in the urothelium of carcinogen- and/or retinoid-treated animals. Any relative incidental toxicities of the retinoids will be assessed histologically.

Major Findings: It is too soon to observe the effect of 13-*cis*-retinoic acid on the neoplastic response of the urothelium to OHBBN since this is a new project. The growth curves of the animals indicate the dose of retinoid used is well tolerated. The initial response of the urothelium to the OHBBN indicates that there has been preneoplastic transformation of the urothelium of all animals examined so far.

Approximately 250 rats have received a full carcinogenic treatment with the selective bladder carcinogen hydroxybutylbutanol nitrosamine (OHBBN) and these and control untreated animals are now being maintained either on a 13-*cis*-retinoic acid containing diet or a placebo diet. These animals are being sequentially sampled for histological and ultrastructural tissue assessment. Other groups (altogether a further 350 animals) are currently receiving carcinogen treatment and these animals will be maintained on different concentrations of another retinoid (retinoic acid ethylamide) for a life-time assessment of their response.

Significance to Biomedical Research and the Program of the Institute: The results obtained so far confirm the viability of the project and show the model we are using has the potential to produce the information required.

Proposed Course: To continue the studies as outlined in the investigative protocol.

Date Contract Initiated: September 30, 1977

Current Annual Level: $81,169

NATIONAL ACADEMY OF SCIENCES/INSTITUTE OF LABORATORY ANIMAL RESOURCES (N01-CP-65805)

Title: Histologic Classification of Laboratory Animal Tumors

Contractor's Project Director: Dr. Samuel Abramson

Project Officer (NCI): Dr. Morton H. Levitt

Objectives: To formulate a histological classification and nomenclature for laboratory animal tumors.

Major Findings: The ILAR assembled a committee of experts to review current programs and publications dealing with histologic classification and nomenclature of laboratory animal tumors. It was the committee's findings that the present programs do not fully meet the needs for standardized terminology and histologic classification of laboratory animal tumors. After a discussion

of priority of needs it was decided that rat liver tumors should be the first subject of an effort to develop a tentative histologic classification and nomenclature. The committee further concluded that it would be in the best interests of the study if a subcommittee consisting of pathologists intimately involved in the pathology of rat liver tumors be appointed to develop the report. The subcommittee was appointed in December, 1976.

The subcommittee developed a draft document entitled "Histologic Typing of Liver Tumors of the Rat" that includes both benign and malignant tumors and associated lesions as well as pertinent references. The material covered in the draft is illustrated by color photographs. After this draft receives final editing, peer review by a group of consultant pathologists who will attempt to apply the proposed classification to typing of rat liver tumors in their personal collections, and final review and approval by the National Research Council, it is proposed to submit it for publication in the Journal of the National Cancer Institute.

Significance to Biomedical Research and the Program of the Institute: There is within the biomedical community a lack of uniformity in identifying and classifying pathological lesions, particularly laboratory animal tumors as they relate to the tumors in man. The frequency and nature of tumors in the body sites differ markedly among the various species, breeds, and regions. The histologic terminology applied to these pathological lesions is used loosely; in many published reports, it is not clear whether investigators in different laboratories are observing the same tumors, or whether uniform nomenclature is being used to describe similar tumors in different species. Articles on the histology and classification of tumors tend to be widely scattered in the scientific publications; many are not easily obtainable. This circumstance, in the face of a continuing rise in the number of compounds being tested for carcinogenicity (wherein carcinogenicity is determined on the basis of tumors found in experimental animals), makes it imperative that the histologic classification and nomenclature of laboratory animal tumors be standardized.

Proposed Course: This contract has been allowed to come to an orderly expiration. Future undertakings on histologic classification and nomenclature of laboratory animal tumors will be initiated as appropriate.

Date Contract Initiated: June 30, 1976

Current Annual Level: $40,000

TEXAS, UNIVERSITY OF (N01-CP-65846)

Title: The Biology of Neoplastic Liver Lesions in Mice

Contractor's Project Director: Dr. Frederick F. Becker

Project Officer (NCI): Dr. Sherman F. Stinson

Objectives: The major objective of this program is to develop objective means of characterizing spontaneous and chemically induced mouse liver lesions in C3H and C57BL/6 inbred mouse strains.

Major Findings: The rate of spontaneous hepatocellular carcinoma (PHC) development in the C3H males when raised in the contractor's laboratory is approximately 64% at one year and in excess of 85% at fifteen months. In all instances these tumors were multiple and ranged in size from 1 mm to 2.5 cm in diameter. It was demonstrated that some factor in the prior environment of retired breeder males obtained from the same colony induced a significant delay in onset of their PHC such that less than 5% demonstrated tumors at one year and 27% were evident at fifteen months. In addition, these were less numerous and had a smaller average diameter.

Serum AFP levels were followed throughout the life of the C3H mice. In the absence of PHC's, no significant or consistent (through two months of determination) elevation of AFP was found in any mouse. The detection of a significant elevation, defined as an elevation which progressed through two determinations at a month's interval, was invariably associated with PHC's. Due to the multiplicity of most tumors it was difficult to specifically identify the source of this elevation but a rough correlation between its amplitude and the total tumor mass was evident. However, preliminary immunofluorescent studies have indicated that all tumors thus far studied demonstrate at least some population of AFP-positive cells.

No spontaneous PHC's have been demonstrated in the C57BL/6 mice. They have demonstrated a truly remarkable resistance to the two chemical carcinogens tested thus far. After one year of exposure to "significant" levels of acetylaminofluorene (AAF) up to 0.06%; and to chlordane, up to 50 ppm, no identifiable liver lesion has been demonstrated nor has there been induced any alteration in serum AFP.

The chromatin proteins of spontaneous PHC in C3H/N mice, the "background liver" from these mice, and from young, non-tumor-bearing mice of the same strain have been isolated, purified and characterized. Significant differences in the banding pattern of the PHC from those of background and non-tumor-bearing animals has been evident.

Representative PHC from each of the above experiments have been transplanted into compatible mice in multiple locations. To date, only a suggestion of evidence of growth has been obtained confirming previous studies which showed such tumors to be extremely slow growing and requiring as long as one year to appear.

Initial exposure of C3H/N mice to both AAF and chlordane demonstrated significant differences between this strain and that of the C57. C3H showed a greatly increased susceptibility to the toxic and lethal effects of AAF while apparently being equally resistant to chlordane. Apparently, the

C3H mouse demonstrates an extremely sharp threshold for the toxic effects of AAF. On the basis of past studies this susceptibility would suggest that carcinogenesis will follow prolonged exposure.

Significance to Biomedical Research and the Program of the Institute: Since the mouse is one of the most commonly used animals in carcinogenesis research, including long term bioassay, an understanding of the biology of neoplastic liver lesions in this species is important. Comparison of biologic properties of neoplastic liver lesions should lead to the determination of whether unifying criteria for classification exist.

Proposed Course: To continue the evaluation of induced and spontaneous lesions in the mouse liver. Suggestions exist that the C57 mouse may begin to show lesions during the second year of exposure. Such animals are already entering that year and are under observation both morphologically and by AFP evaluation. The susceptibility of the C3H mouse to the toxic qualities of AAF suggests that this agent will induce lesions in this strain. One of the first major tasks will be to compare those lesions induced by chemical agents with the lesions that arise spontaneously in such mouse strains. The identification of these induced lesions as malignant by transplantability and other criteria, and the ability to distinguish them from those which are genetically determined should be a major part of the task in the coming year.

Lectin agglutination studies of the mouse will be applied to the mouse liver lesions as they become manifest.

Chromatin studies are continuing with the application of more advanced analytic techniques associated with laser technology to the automatic analysis of the chromatin protein bands. Of particular importance will be the comparison of these components in chemically induced tumors with those which arise spontaneously.

Date Contract Initiated: September 30, 1976

Current Annual Level: $145,227

WISCONSIN, UNIVERSITY OF (at Madison) (NO1-CP-75905)

Title: Long Term Studies of Prevention of Epithelial Cancer by Retinoids

Contractor's Project Director: Dr. William A. Croft

Project Officers (NCI): Dr. Michael B. Sporn
Dr. Carl C. Smith
Dr. Morton H. Levitt

Objectives: The object of this program is to acquire information concerning the effect of synthetic retinoids on the development of urinary bladder cancer in experimental animals. The present study is aimed at obtaining unequivocal

data for the prevention of bladder cancer during the neoplastic period by different retinoic compounds in rats. An experimental model for the induction of rat bladder cancer by N-[4-(5-nitro-2-furyl)-2-thiazolyl]-formamide (FANFT) has been developed in the laboratory. This model will provide a good opportunity for the determination of the efficacy of retinoic compounds in the prevention of bladder cancer during the preneoplastic period.

Major Findings: Since January 9, 1978, 965 rats of ten different groups were placed on study using FANFT as the carcinogen, and 13-*cis* retinoic acid as the retinoid. Placebo is used as carrier of the retinoid. At the time of this writing, the 13th week of the study, no gross evidence of tumors has been detected, but the animals are scheduled to go for 50 weeks. At the end of the study, each rat will be evaluated for bladder weight, number of tumors per bladder detected grossly, percent urinary bladder having hyperplasia, papilloma, and carcinoma. Histological evaluation of each lesion will be based on the classification of the World Health Organization.

Significance to Biomedical Research and the Program of the Institute: The literature has demonstrated potential use of retinoids in inhibition of cancer in man and animals. This study will provide data concerning the efficacy of 13-*cis* retinoic acid in the inhibition of urinary bladder cancer induced by FANFT. Retinoids, then, if found to be effective in such studies, could be used in a chemoprevention program aimed at inhibiting the formation of urinary bladder cancer in the preneoplastic stage and reducing the incidence of bladder cancer in man until better modes of prevention or therapy can be established.

Proposed Course: A continuation of the studies as outlined above.

Date Contract Initiated: September 1, 1977

Current Annual Level: $130,000

NARRATIVES OF CONTRACTS IMPLEMENTED AT THE END OF FISCAL YEAR 1978

ALABAMA, UNIVERSITY OF (at Birmingham) (NO1-CM-67083)

Title: Rodent Disease Surveillance (Supplement to Division of Cancer Treatment contract)

Contractor's Project Director: Dr. J. Russell Lindsey

Project Officers (NCI): Dr. Thomas Cameron
Dr. Joseph Mayo

Objectives: To monitor the health status of the rodents as they are produced by the present production contractors as well as when those animals are used by the testing laboratories for bioassay studies.

Major Findings: None, as this supplementary project has only recently been implemented.

Significance to Biomedical Research and the Program of the Institute: The animals produced for the Carcinogenesis Testing Program must be maintained as virologically, bacteriologically and parasitologically free as possible in order to achieve the highest possible survival rates. To achieve this goal, all breeders that are placed in barrier-protected production rooms originate from associated flora plastic isolators and are used in the testing facilities that employ optimal husbandry practices.

Proposed Course: Monthly random samples of animals, representing the spectrum of animal colonies and the individual rooms of the production contracts will be examined alternately by this contractor and another facility under contract to the Division of Cancer Treatment, NCI, for pathological, bacteriological and parasitological aspects and for their serological profile each month at a third laboratory under contract to the Division of Cancer Treatment. In addition, forty animals of each species will be entered into each chronic compound study at its inception. These animals will be examined periodically for serological profiles. The result will be a complete, detailed health history of the animals as they progress from production through the test procedures. The CGT Program staff will thus be able to monitor: 1) the ability of the respective animal producers to breed and issue animals of the highest health status consistently; and, 2) the relative abilities of the various testing laboratories to maintain animals free of infections--in addition, corrective measures can be recommended and instituted as necessary as problems are identified. As a result, the health history of the test animals can be entered as an integral component of the technical report that summarizes the results of the compound test.

Date Contract Initiated: June 30, 1978 (supplement only)

Current Annual Level: $100,000 (supplement only)

V. PUBLICATIONS

A. Carcinogenesis Bioassay Reports

The published Carcinogenesis bioassay reports may be purchased from the National Technical Information Service, U.S. Department of Labor, 5285 Port Royal Road, Springfield, Virginia 22161 (telephone 703 557-4650) or obtained from the Office of Cancer Communications as long as their supply lasts. Reports on the following chemicals are available:

Technical Report Number	Chemical and CAS Number	DHEW Publication Number	NTIS Accession Number
	chlordecone CAS No. 143-50-0		PB264041/AS
	chloroform CAS No. 67-66-3		PB264018/AS
1	(Guidelines for Carcinogen Bioassay in Small Rodents)	(NIH)76-801	PB264061/AS
2	trichloroethylene CAS No. 79-01-6	(NIH)76-802	PB264122/AS
3	1,1,1-trichloroethane CAS No. 71-55-6	(NIH)77-803	PB265082/AS
4	dimethoate CAS No. 60-51-5	(NIH)77-804	PB264367/AS
5	proflavine CAS No. 952-23-8	(NIH)77-805	PB268553/AS
6	nitrilotriacetic acid (NTA) and nitrilotriacetic acid, trisodium salt, monohydrate ($Na_3NTA \cdot H_2O$) CAS No. 139-13-9 (NTA) CAS No. 18662-53-8 ($Na_3NTA \cdot H_2O$)	(NIH)77-806	PB266177/AS
7	phenformin CAS No. 114-86-3	(NIH)77-807	PB266176/AS
8	chlordane CAS No. 57-74-9	(NIH)77-808	PB271977/AS
9	heptachlor CAS No. 76-44-8	(NIH)77-809	PB271967/AS
10	dichlorvos CAS No. 62-73-7	(NIH)77-810	PB270937/AS

Technical Report Number	Chemical and CAS Number	DHEW Publication Number	NTIS Accession Number
11	trisodium ethylenediaminetetra-acetate trihydrate (EDTA) CAS No. 150-38-9	(NIH)77-811	PB270938/AS
13	tetrachloroethylene CAS No. 127-18-4	(NIH)77-813	PB272940/AS
14	lindane CAS No. 58-89-9	(NIH)77-814	PB273480/AS
15	captan CAS No. 133-06-2	(NIH)77-815	PB273475/AS
17	photodieldrin CAS No. 13366-73-9	(NIH)77-817	PB274393/AS
18	3,3'-iminobis-1-propanol dimethanesulfonate (ester) hydrochloride (IPD) CAS No. 3458-22-8	(NIH)77-818	PB277455/AS
20	dapsone CAS No. 80-08-0	(NIH)77-820	PB274394/AS
21	aldrin and dieldrin CAS Nos. 309-00-2 and 60-57-1	(NIH)77-821	PB275666/AS
22	dieldrin CAS No. 60-57-1	(NIH)77-822	PB275676/AS
23	picloram CAS No. 1918-02-1	(NIH)77-823	PB276471/AS
24	malathion CAS No. 121-75-5	(NIH)77-824	PB278527/AS
25	chloramben CAS No. 133-90-4	(NIH)77-825	PB273065/AS
26	nitrofen CAS No. 1836-75-5	(NIH)78-826	PB277440/AS
27	1,1,2,2-tetrachloroethane CAS No. 79-34-5	(NIH)78-827	PB277453/AS
28	dibromochloropropane CAS No. 1836-75-5	(NIH)78-828	PB277472/AS

Technical Report Number	Chemical and CAS Number	DHEW Publication Number	NTIS Accession Number
29	2-methyl-1-nitroanthraquinone CAS No. 129-15-7	(NIH)78-829	PB277439/AS
30	diarylanilide yellow CAS No. 6358-85-6	(NIH)78-830	PB278272/AS
31	tolbutamide CAS No. 64-77-7	(NIH)78-831	PB274483/AS
32	isophosphamide CAS No. 3778-73-2	(NIH)78-832	PB275677/AS
33	tetrachlorvinphos CAS No. 961-11-5	(NIH)78-833	PB278650/AS
34	trifluralin CAS No. 1582-09-8	(NIH)78-834	PB278610/AS
35	methoxychlor CAS No. 72-43-5	(NIH)78-835	PB278271/AS
36	anthranilic acid CAS No. 118-92-3	(NIH)78-836	PB278883/AS
38	aroclor[R] 1254 CAS No. 27323-18-8	(NIH)78-838	
39	lasiocarpine CAS No. 303-34-4	(NIH)78-839	PB278641/AS
40	hexachlorophene CAS No. 70-30-4	(NIH)78-840	
42	5-azacytidine	(NIH)78-842	
43	emetine CAS No. 483-18-1	(NIH)78-843	PB278891/AS
44	(*In Vitro Carcinogenesis*)	(NIH)78-844	
45	chlorpropamide CAS No. 94-20-2	(NIH)78-845	PB275178/AS
47	4,4'-thiodianiline CAS No. 139-65-1	(NIH)78-847	
48	pyrazinamide CAS No. 98-96-4	(NIH)78-848	

Technical Report Number	Chemical and CAS Number	DHEW Publication Number	NTIS Accession Number
52	3-nitropropionic acid CAS No. 504-88-1	(NIH)78-1302	
54	2,4-dinitrotoluene CAS No. 121-14-2	(NIH)78-1360	
56	N,N'-dicyclohexylthiourea CAS No. 1212-29-9	(NIH)78-1362	
57	beta-TGdr CAS No. 789-61-7	(NIH)78-1363	
76	tris(2,3-dibromopropyl) phosphate CAS No. 126-72-7	(NIH)78-1326	
84	2,4-diaminoanisole sulfate CAS No. 615-05-4	(NIH)78-1334	
108	direct blue 6, direct black 38, and direct brown 95 dyes CAS Nos. 2602-46-2, 1937-37-7, 16071-86-6	(NIH)78-1358	

B. Other Publications

1. Agthe, C. and Tomatis, L.: Evaluation of the Carcinogenicity of environmental chemicals and the possibility of cancer prevention. In Homburger, F. (Ed.): The Physiopathology of Cancer. Vol. II - Diagnosis, Treatment, Prevention. Basel, S. Karger, 1976, pp. 345-358.

2. Bartsch, H.: The use of microbial mutagenicity assays for the detection of chemical carcinogens. In Mécanismes d'altération et de réparation du DNA, rélations avec la mutagénèse et la cancérogénèse chimique. Colloques internationaus du CNRS, No. 256, 1977, pp. 255-272.

3. Bartsch, H. and Loprieno, N.: Vinyl chloride (VCM): An example for evaluating adverse biological effects in short-term tests. In Böhme, H. and Schöneich, J. (Eds.): Proceedings of the 6th EEMS-Meeting. Gernrode, Abhandlung der Akademie der Wissenschaffen, 1977, pp. 162-165.

4. Bartsch, H., Malaveille, C., Stich, H.F., Miller, E.C., and Miller, J.A.: Comparative electrophilicity, mutagenicity, DNA repair induction activity and carcinogenicity of some N- and O-acyl derivatives of N-hydroxy-2-aminofluorene. Cancer Res. 37: 1461-1467, 1977.

5. Bartsch, H., Margison, G.P., Malaveille, C., Camus, A.M., Brun, G., and Margison, J.M.: Some aspects of metabolic activation of chemical carcinogens in relation to their organ specificity. Arch. Toxicol (Berl.) 39: 51-63, 1977.

6. Berman, J., Rice, J.R., and Strandberg, J.D.: Granular cell variants in a rat schwannoma, evidence of neurogenic origin of granular cell tumors (myoblastomas). Vet. Pathol. (In press).

7. Berman, J.J., Tong, C., and Williams, G.M.: Enhancement of mutagenesis during cell replication of cultured liver epithelial cells. Cancer Lett. (In press).

8. Blakeslee, J. and Milo, G.: Feline sarcoma virus in vitro infection of human cells. Influence of chemical carcinogens on focus formation. Chem. Biol. Interact. (In press).

9. Bouck, N., and di Mayorca, G.: In vitro neoplastic transformation of BHK cells. Methods of Enzymol. (In press).

10. Bouck, N. and Di Mayorca, G.: Somatic mutation as the basis for in vitro transformation of BHK cells by chemical carcinogens. In Saffiotti, U. and Autrup, H. (Eds.): In Vitro Carcinogenesis - Guide to the Literature, Recent Advances and Laboratory Procedures. U.S. DHEW, PHS, NIH, NCI. DHEW Publ. No. (NIH)78-844. Wash., D.C., U.S. Govt. Print. Off., 1978, pp. 76-81.

11. Brownstein, D.G.: Pathogenesis of pseudomonas aeruginosa bacteremia in cyclophosphamide treated mice and potentiation of endogenous streptococcal virulence. J. Infect. Dis. (In press).

12. Brownstein, D.G., Strandberg, J.D., Montali, R.J., Bush, R.M., and Fortner, J.: Cryptosporidium in snakes with hypertrophic gastritis. Vet. Pathol. 14: 606-617, 1977.

13. Camus, A.M., Wiessler, M., Malaveille, C., and Bartsch, H.: High mutagenicity of N-(αacyloxy)alkyl-N-alkylnitrosamines in S. typhimurium: Model compounds for metabolically activated N,N-dialkylnitrosamines. Mutat. Res. 49: 187-194, 1978.

14. Cardesa, A., Bullon-Ramirez, A., and Levitt, M.: Tumours of the pancreas. In Turusov, V.S. (Ed.): Pathology of Tumours in Laboratory Animals. Vol. II - Tumours of the Mouse. IARC Scientific Publications No. 7 (In press).

15. Cardy, R.H., Gilmore, C., Renne, R.A., Warner, J.W., and Bypher, R.L.: The results of a carcinogenesis bioassay of piperonyl sulfoxide in Fischer 344 rats and B6C3F1 hybrid mice. J. Natl. Cancer Inst. (In press).

16. Cardy, R.H., Gilmore, C., Warner, J.W., and Cypher, R.L.: Carcinogensis bioassay of piperonyl butoxide in B6C3F1 hybrid mice. J. Natl. Cancer Inst. (In press).

17. Cardy, R.H., Renne, R.A., and Warner, J.W.: Carcinogenesis of piperonyl butoxide in Fischer 344 rats. J. Natl. Cancer Inst. (In press).

18. Casto, B.C.: Enhancement of DNA viral transformation by chemical carcinogens and mutagens. In Saffuotti, U. and Autrup, H. (Eds.): In Vitro Carcinogenesis - Guide to the Literature, Recent Advances, and Laboratory Procedures. U.S. DHEW, PHS, NIH, NCI. DHEW Publ. No. (NIH)78-844. Wash., D.C., U.S. Govt. Print. Off., 1978, pp. 226-230.

19. Casto, B.C.: In vitro testing for carcinogensis: Agreement (or lack thereof) among current methods of their correlation with animal studies. In Berky, J. and Sherrod, C. (Eds.): Report from the Committee on Carcinogenesis, Mutagenesis, and Toxicity Testing In Vitro - In Vitro Toxicity Testing 1975/1976. Philadelphia, The Franklin Institute Press, 1978, pp. 373-392.

20. Casto, B.C.: In vitro testing for chemical carcinogens: Transformation assays in mammalian cells. In Vitro 13: 191-192, 1977.

21. Casto, B.C.: Oncogenic transformation of mammalian cells by chemicals and chemical-viral interactions. In Waters, M.D. (Ed.): Proceedings Symposium on Application of Short-term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures (In press).

22. Casto, B.C. and DiPaolo, J.A.: Biological activity of polycyclic hydrocarbons for Syrian hamster cells in vitro. In Gelboin, H.V. and Ts'o, P.O.P. (Eds.): Polycyclic Hydrocarbons and Cancer: Chemistry, Molecular Biology and Environment (In press).

23. Casto, B.C. and Hatch, G.G.: Developments in neoplastic transformation. In Berky, J. and Sherrod, C. (Eds.): Report from the Committee on Carcinogenesis, Mutagenesis, and Toxicity Testing in Vitro - In Vitro Toxicity Testing, 1975-1976. Philadelphia, The Franklin Institute Press, 1978, pp. 192-214.

24. Casto, B.C. and Janosko, N., and DiPaolo, J.A.: Development of a focus assay model for transformation of hamster cells in vitro by chemical carcinogens. Cancer Res. 37: 3508-3515, 1977.

25. Davies, R.E.: Interaction of light and chemicals in carcinogenesis. J. Natl. Cancer Inst. (In press).

26. DiPaolo, J.A. and Casto, B.C.: In vitro carcinogenesis with cell strains in early passages: In Sanford, K. (Ed.): Gene Expression and Regulation in Cultured Cells (Third Decennial Review Conference on Cell Tissue and Organ Cultures). National Cancer Institute Monograph No. 48 (In press).

27. DiPaolo, J.A. and Casto, B.C.: In vitro transformation of mammalian cells: Sequential treatment with diverse agents. In Slaga, T.J., Boutwell, R.K., Sivak, A. (Eds.): Mechanisms of Tumor Promotion and Cocarcinogenesis (In press).

28. Drevon, C., Kuroki, T., and Montesano, R.: Microsome-mediated mutagenesis of a Chinese hamster cell line by various chemicals. In Scott, D., Bridges, B.A., and Sobels, F.H. (Eds.): Progress in Genetic Toxicology. Amsterdam, Elsevier/North-Holland Biomedical Press, 1977, pp. 207-213.

29. Dunkel, V.C., Wolff, J.S., III, and Pienta, R.J.: In vitro transformtion as a presumptive test for detecting chemical carcinogens. Cancer Bull. 29: 167-174, 1977.

30. Dunn, T.B.: Tumors of the adrenal gland in the mouse. In Turusov, V.S. (Ed.): Pathology of Tumours in Laboratory Animals. Vol. II - Tumours of the Mouse. IARC Scientific Publications No. 7 (In press).

31. Dunn, T.B.: Tumors of the parathyroid gland in the mouse. In Turusov, V.S. (Ed.): Pathology of Tumours in Laboratory Animals. Vol. II - Tumours of the Mouse (In press).

32. Ekelman, K. and Milo, G.: Cellular uptake, transport and macromolecular binding of benzo[a]pyrene and 7,12-dimethylbenz[a]anthracene by human cells in vitro. Cancer Res. (In press).

33. Ekelman, K. and Milo, G.: Transport of polynuclear hydrocarbons into the nucleus of human foreskin cells. In Vitro 13: 183, 1977.

34. Forbes, P.D.: Light sources for solar simulation on photocarcino-carcinogenesis. J. Natl. Cancer Inst. (In press).

35. Forbes, P.D., Davies, R.E., and Urbach, F.: Experimental UV photocarcinogenesis: Wavelength interactions and time-dose relationships. J. Natl. Cancer Inst. (In press).

36. Freeman, A.E., Lake, R.S., Igel, H.J., Gernand, L., Pezzutti, M.R., Malone, J.M., Mark, C., and Benedict, W.F.: Heterploid conversion of human skin cells by methylcholanthrene: Possible role of hydrocarbon metabolizing epithelial cells. In Saffiotti, U. and Autrup H. (Eds.): In Vitro Carcinogenesis - Guide to the Literature, Recent Advances and Laboratory Procedures. U.S. DHEW, PHS, NIH, NCI. DHEW Publ. No. (NIH)78-844. Wash. D.C., U.S. Govt. Print. Off., 1978, pp. 100-122.

37. Goodman, D.G. and Altman, N.H.: Table on spontaneous tumors in inbred rats. In Altman, P.L. and Katz, D.D. (Eds.): Inbred and Genetically Defined Strains of Laboratory Animals Handbook (In press).

38. Grisham, J.W., Charlton, R.K., and Kaufman, D.G.: In vitro assay of cytotoxicity with cultured liver: Accomplishments and possibilities. Environ. Health Perspect. (In press).

39. Gutter, B., Speck, W.T., and Rosenkranz, H.S.: A study of the photoinduced mutagenicity of methylene blue. Mutat. Res. 44: 177-182, 1977.

40. Hatch, G., Balwierz, P., and Casto, B.: Evaluation of in vitro tests for malignancy of chemically-transformed hamster cells. In Vitro 13: 182, 1977.

41. Hatch, G.G., Balwierz, P.J., Casto, B.C., and DiPaolo, J.A.: Characteristics of hamster cells transformed by the combined action of chemical and virus. Int. J. Cancer 21: 121-127, 1978.

42. Hofinger, A.J. and Klopman, G.: A QSAR study of nitrosamine carcinogenicity using aqueous, 1-octanol solvation free energies and the associated partition coefficient as correlation features. Chem. Biol. Interact. (In press).

43. International Agency for Research on Cancer: Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man - Some Fumigants, the Herbicides 2,4-D and 2,4,5-T, Chlorinated Dibenzodioxins and Miscellaneous Industrial Chemicals. Lyon, International agency for Research on Cancer, 1977, Vol. 15, 354 pp.

44. International Agency for Research on Cancer: Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man - Some Aromatic Amines and Related Nitro Compounds--Hair Dyes, Colouring Agents and Miscellaneous Industrial Chemicals. Lyon, International Agency for Research on Cancer, 1978, Vol. 16, 400 pp.

45. Kapadia, G.J., Chung, E.B., Ghosh, B., Shukla, Y.N., Morton, J.F., and Pradhan, S.N.: Carcinogenicity of some folk medicinal herbs in rats. J. Natl. Cancer Inst. 60: 683-684, 1978.

46. Kapadia, G.J., Shukla, Y.N., Morton, J.F., and Lloyd, H.A.: New cyclopeptide alkaloids from Melochia tomentosa. Phytochemistry 16: 1431-1433, 1977.

47. Kennedy, A.R., Mondal, S., Heidelberger, C., and Little, J.B.: Enhancement of x-ray transformation by 12-O-tetradecanoyl-phorbol-13-acetate in a cloned line of C3H mouse embryo cells. Cancer Res. 38: 439-443, 1978.

48. Koziol, J.A., Reid, R., and Levitt, M.H.: A simple non-parametric test for trend: An example based on a biological model for cancer of the pancreas. Biom. Zeit. (In press).

49. Kuroki, T. and Drevon, C.: Driect of proximate contact between cells and metabolic activation systems is required for mutagenesis. Nature 271: 368-370, 1978.

50. Kuroki, T., Drevon, C. and Montesano, R. Microsome-mediated mutagenesis in V79 Chinese hamster cells by various nitrosamines. Cancer Res. 37: 1044-1050, 1977.

51. Kuroki, T., Drevon, C., Saint Vincent, L., Tomatis, L., and Montesano, R.: Studies on the use of liver parenchymal cells in in vitro carcinogenesis. In Mécanismes d'altération et de réparation du DNA, rélations avec la mutagénèse et la cancérogénèse chimique. Colloques internationaux du CNRS, No. 256, 1977, pp. 307-314.

52. Lake, R.S., Freeman, A.E., Igel, H.J., Pezzutti, M.R., and Kropko, M.L.: In vitro culture of diploid human skin epithelial cells for chemical transformation studies. In Saffiotti, U. and Autrup H. (Eds.): In Vitro Carcinogenesis - Guide to the Literature, Recent Advances and Laboratory Procedures. U.S. DHEW, PHS, NIH, NCI. DHEW Publ. No. (NIH)78-844. Wash., D.C., U.S. Govt. Print. Off., 1978, pp. 283-291.

53. Lake, R.S., Kropko, M.L., Pezzutti, M.R., Shoemaker, R.H., and Igel, H.J.: Chemical induction of unscheduled DNA synthesis in human skin epithelial cell cultures. Cancer Res. (In press).

54. Levitt, M.H., Harris, C.C., Squire, R., Wenk, M., Mollelo, C., and Springer, S.: Experimental pancreatic carcinogenesis. II. Lifetime carcinogenesis studies in the outbred Syrian golden hamster with N-nitrobis(2-hydroxypropyl)amine. J. Natl. Cancer Inst. 60: 701-705, 1978.

55. Likhachev, A.J., Margison, G.P., and Montesano, R.: Alkylated purines in the DNA of various rat tissues after administration of 1,2-dimethylhydrazine. Chem. Biol. Interact. 18: 235-240, 1977.

56. Loew, G.H. Phillips, J., Wong, J., Hjelmeland, L., and Pack, G.R.: Quantum chemical studies of the metabolism of polycyclic aromatic hydrocarbons: Bay region reactivity as a criterion for carcinogenic potency. Cancer Biochem. Biophys. (In press).

57. Lowe, G.H., Wong, J., Phillips, J., Hjelmeland, L., and Pack, G.R.: Quantum chemical studies of the metabolism of benzo[a]pyrene. Cancer Biochem. Biophys. (In press).

58. Loprieno, N., Barale, R., Baroncelli, S., Bartsch, H., Bronzetti, G., Cammellini, A., Corsi, C., Frezza, D., Nieri, R., Leporini, C., Rosellini, D., and Rossi, A.M.: Induction of gene mutations and gene conversions by vinyl chloride metabolites in yeast. Cancer Res. 36: 253-257, 1977.

59. Malaveille, C., Kuroki, T., Sims, P., Grover, P.L., and Bartsch, H.: Mutagenicity of isomeric diol-epoxides of benz[a]pyrene and benz[a]anthracene in S. typhimurium TA 98 and TA100 and in V79 Chinese hamster cells. Mutat. Res. 44: 313-326, 1977.

60. Malaveille, C., Planche, G., and Bartsch, H.: Factors for efficiency of the Salmonella/microsome mutagenicity assay. Chem. Biol. Interact. 17: 129-136, 1977.

61. Malaveille, C., Tierney, B., Grover, P.L., Sims, P., and Bartsch, H.: High microsome-mediated mutagenicity of the 3,4-dihydrodiol of 7-methylbenz-[a]anthracene in S. typhimurium TA98. Biochem, Biophys. Res. Commun. 75: 427-433, 1977.

62. Margison, G.P., Margison, J.M., and Montesano, R.: Accumulation of O^6-methylguanine in non-target-tissue deoxyribonucleic acid during chronic administration of dimethylnitrosamine. Biochem. J. 165: 563-468, 1977.

63. Milo, G., Blakeslee, J., Hart, R., and Yohn, D.S.: Chemical carcinogen alteration of SV-40 virus induced transformation of normal human cell populations in vitro. Chem. Biol. Interact. (In press).

64. Milo, G., Blakeslee, J., Yohn, D., and DiPaolo, J.: Biochemical activation of AHH activity, cellular distribution of polynuclear hydrocarbon metabolites and DNA damage by PNH products in human cells in vitro. Cancer Res. 38: 1638-1644, 1978.

65. Mishra, N.K., Pant, K., and Wilson, C.M.: Carcinogen-induced cellular mutation rates at two genetic loci are not affected by co-carcinogenic leukemia virus infection. Nature 266: 548-550, 1977.

66. Mishra, N.K., Wilson, C.M., Pant, K.J., and Thomas, F.O.: Simultaneous determination of cellular mutagenesis and transformation by chemical carcinogens in FRE cells. J. Toxicol. Environ. Health 4: 79-91, 1978.

67. Mondal, S. and Heidelberger, C.: Role of ultraviolet light in the oncogenic transformation of cultured C3H/10T1/2 mouse embryo cells. In Hanna, M.G., Jr. and Kripke, M.: International Conference on Ultraviolet Carcinogenesis. National Cancer Institute Monograph No. 50 (In press).

68. Montesano, R., Drevon, C., Kuroki, T., Saint Vincent, L., Handleman, S., Sanford, K.K., DeFeo, D., and Weinstein, I.B.: Test for malignant transformation of rat liver cells in culture: Cytology, growth in soft agar andproduction of plasminogen activator. J. Natl. Cancer Inst. 59: 1651-1658, 1977.

69. Montesano, R., Margison, G.P., and Likhachev, A.: The possible role of nucleic acid alkylation in the organ specificity of carcinogens. In Farber, E., Kawachi, T., Nagayo, T., Sugano, H., Sugimura, T., and Weisburger, J.H. (Eds.): Pathophysiology of Carcinogenesis in Digestive Organs. Tokyo/Baltimore, University of Tokyo Press/University Park Press, 1977, pp. 221-231.

70. Mugera, G.M. and Ward, J.M.: Acute toxicity of maytansine in F344 rats. Cancer Treat. Rep. 61: 1333-1338, 1977.

71. Munoz, N. and Dunn, T.B.: Tumours of the uterus in mice. In Turusov, V.S. (Ed.): Pathology of Tumours in Laboratory Animals. Vol. II Tumours of the Mouse (In press).

72. Murthy, A.S.K., Baker, J.R., Smith, E.R., and Wade, G.G.: Development of hemangiosarcomas in B6C3F1 mice, red 2-methyl-1-nitroanthraquinone. Int. J. Cancer 19: 117-121, 1977.

73. McCoy, E.C., Hankel, R., Robbins, K. Rosenkranz, H.S., Giuffrida, J.G., and Bizzari, D.R.: Presence of mutagenic substances in the urine of anesthesiologists. Mutat. Res. (In press).

74. McCoy, E.C., Hankel, R., Rosenkranz, H.S., Giuffrida, J.G., and Bizzari, V.: Detection of mutagenic activity in the urines of anesthesiologists: A preliminary report. Environ. Health. Perspect. (In press).

75. McCoy, E.C., Speck, W.T., and Rosenkranz, H.S.: Activation of a procarcinogen to a mutagen by cell-free extracts of anaerobic bacteria. Mutat. Res. 46: 261-264, 1977.

76. Noonan, K.D., Bouck, N., and di Mayorca, G.: The dissociation of the surface architecture described by enhanced lectin agglutinability and the transformed phenotype expressed as anchorage independe. J. Cell. Physiol. 91: 201-207, 1977.

77. Pack, G.R. and Loew, G.H.: Origins of the specificity in the intercalation of ethidium in nucleic acids: A theoretical analysis. Biochem. Biophys. Act. (In press).

78. Pack, G.R., Loew, G.H., Yamabe, S., and Morokuma, K.: A comparative study of semiempirical methods for calculating interactions between large molecules. Int. J. Quant. Chem. Quant. Biol. Sym. (In press).

79. Pedersen, N.C., Ward, J.M., and Mengeling, W.L.: Antigenic relationship of the feline infectious peritonitis virus to corona virus of other species. Arch. Virol. (In press).

80. Peterson, A., Mondal, S., Brankow, D., Thon, W., and Heidelberger, C.: Effects of promoters on DNA synthesis in C3H/10T1/2 mouse fibroblasts. Cancer Res. 37: 3223-3227, 1977.

81. Pienta, R.J.: The use of early passage hamster embryo cells for the identification of chemical carcinogens. In Saffiotti, U. and Autrup, H. (Eds.): In Vitro Carcinogenesis - Guide to the Literature, Recent Advances and Laboratory Procedures. U.S. DHEW, PHS, NIH, NCI, DHEW Publ. No. (NIH)78-844. Wash., D.C., U.S. Govt. Print. Off., 1978, pp 69-75.

82. Pienta, R.J.: Workshop on Laboratory Procedures. The use of early passage hamster embryo cells for the identification of chemical carcinogens. In Saffiotti, U. and Autrup, H. (Eds.): In Vitro Carcinogenesis - Guide to the Literature, Recent Advances and Laboratory Procedures. U.S. DHEW, PHS, NIH, NCI, DHEW Publ. No. (NIH)77-844. Wash., D.C., U.S. Govt. Print. Off., 1978, pp. 273-277.

83. Pienta, R.J., Poiley, J.A., and Lebherz, W.B. III: Fu-ther evaluation of a hamster embryo cell carcinogenesis bioassay. In Cancer Protection and Detection (In press).

84. Pienta, R.J., Shah, M.J., and Lebherz, W.B.III: Correlation of bacterial mutagenicity and hamster cell transformation with tumorigenicity induced by 2,4-toluenediamine. Cancer Lett. 3: 45-52, 1977.

85. Pienta, R.J., Poiley, J.A., and Lebherz, W.B. III: Morphological transformation of early passage golden hamster embryo cultures as a reliable in vitro bioassay for identifying diverse carcinogens. Int. J. Cancer 19: 642-655, 1977.

86. Poiley, J.A., Schuman, R.F., and Pienta, R.J.: Characterization of normal human embryo cells grown to over 100 population doublings. In Vitro (In press).

87. Pour, P. and Levitt, M.H.: Tumors of the Pancreas, Model No. XXX. In Jones, T.C., Hackel, D.B., and Migaki, G. (Eds.): Animal Models of Human Disease (In press).

88. Priester, W.A., Goodman, D.G., and Theilen, G.H.: Nine simultaneous primary tumors in a boxer dog. J. Am. Vet. Med. Assoc. 170: 823-826, 1977.

89. Raineri, R., Poiley, J.A., Ernst, M.K., Hillesund, T., and Pienta, R.J.: A high pressure liquid chromatography procedure for the separation of metabolites of 2-acetylamino-fluorene from cells in culture. J. Liquid Chromatog. (In press).

90. Rosenkranz, H.S.: E. coli DNA-repair deficient assays. In Comparative Mutagenicity (In press).

91. Rosenkranz, H.S.: The mutagenicity of halogenated alkanes and their derivatives. Environ. Health Perspect. (In press).

92. Rosenkranz, H.S., McCoy, E.C., Anders, M., Speck, W.T., and Bickers, D.: The use of microbial assay systems in the detection of environmental mutagens in complex mixtures. In Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures (In press).

93. San, R.H.C., Rice, J.M., and Williams, G.M.: Lack of correlation between plasminogen activating factor production and tumorigenicity in rat liver epithelial cells. Cancer Lett. 3: 243-246, 1977.

94. San, R.H.C. and Williams, G.M.: Rat hepatocyte primary cell culture - Mediated mutagenesis of adult rat liver epithelial cells by procarcinogens. Proc. Soc. Exp. Biol. Med. 156: 534-538, 1977.

95. Speck, W.T., Santella, R.M., and Rosenkranz, H.S.: An evalution of the prophage λ induction (inductest) for the detection of potential carcinogens. Mutat. Res. (In press).

96. Squire, R.A. and Goodman, D.G.: Tumors in laboratory animals. In Benirschke, K., Garner, F.M., and Jones, T.C. (Eds.): Pathology of Laboratory Animals. New York, Springer-Verlag

97. Squire, R.A., Goodman, D.G., Dawe, C.J., Frederickson, T., Harshbarger, J.C., and Levitt, M.H., Lingeman, C.H., Strandberg, J.D., and Valerio, M.G.: Tumors. In Benirschke, K., Garner, F.M., and Jones, T.C. (Eds.): Pathology of Laboratory Animals (In press).

98. Stewart, H.L.: Enigmas of cancer in relation to neoplasms of aquatic animals. Ann. NY Acad. Sci. 298- 305-315, 1977.

99. Stewart, H.L.: Fathollah Keshvar Mostofi - A Profile. Int. Pathol. A News Bulletin 18 (4): 1-3, 1977.

100. Stewart, H.L.: Tumors of the soft tissues. In Turusov, V.S. (Ed.): Pathology of Tumours in Laboratory Animals. Vol. II - Tumours of the Mouse (In press).

101. Stewart, H.L., Dunn, T.B., Snell, K.C., and Deringer, M.K.: Tumors and proliferative lesions of the respiratory apparatus in mice. In Turusov, V.S. (Ed.): Pathology of Tumours in Laboratory Animals. Vol. II - Tumors of the Mouse (In press).

102. Tomatis, L.: The value of long-term testing for the implementation of primary prevention. In Hiatt, H., Watson, J.D., and Winsten, J. (Eds.): Origins of Human Cancer. Book C - Human Risk Assessment. Cold Spring Harbor, Cold Spring Harbor Laboratory, 1977, pp. 1339-1357.

103. Tomatis, L., Agthe, C., Bartsch, H., Huff, J., Montesano, R., Saracci, R., Walker, E., and Wilbourn, J.: Evaluation of the carcinogenicity of chemicals: A review of the IARC Monograph Programme, 1971-1977. Cancer Res. 38: 877-885, 1978.

104. Ward, J.M.: Pulmonary pathology of the motheaten mouse. Vet. Pathol. 15: 170-178, 1978.

105. Ward, J.M., Rice, J.M., Roller, P.P., and Wenk, M.L.: Natural history of intestinal neoplasms induced in rats by a single injection of methyl (acetoxymethyl) nitrosamine. Cancer Res. 37: 3046-3052, 1977.

106. Ward, J.M., Sporn, M.B., Wenk, M.L., Smith, J.M., Feeser, D., and Dean, R.J.: Dose response to intrarectal administration of N-methyl-n-nitrosourea and histopathologic evaluation of the effect of two retinoids on colon lesions induced in rats. J. Natl. Cancer Inst. (In press).

107. Ward, J.M. and Vlahakis, G.: Evaluation of hepatocellular neoplasms in mice. J. Natl. Cancer Inst. (In press).

108. Wassom, J.S., Huff, J.E., and Loprieno, N.: A review of the genetic toxicology of chlorinated dibenzo-p-dioxins. Mutat. Res. (In press).

109. Wassom, J.S. and Malling, H.V.: Specialized information centers in toxicology: Environmental Mutagen Information Center (EMIC). In Mehlman, M. and Flamm, G.W. (Eds.): Advances in Toxicology: Mutagenesis. Washington, emisphere Publishing Corporation, 1978, pp. 349-383.

110. Waters, R., Bouck, N., di Mayorca, G., and Regan, J.D.: Partial inhibition of postreplication repair and enhanced frequency of chemical transformation in rat cells infected with leukemia virus. Proc. Natl. Acad. Sci. USA 74: 238-242, 1977.

111. Waters, R., Mishra, N., Bouck, N., di Mayorca, G., and Regan, J.C.: Partial inhibition of postreplication repair and enhanced frequency of chemical transformation in rat cells infected with leukemia virus. Proc. Natl. Acad. Sci. USA 74: 238, 1977.

112. Weisburger, E.K., Russfield, A.B., Homburger, F., Weisburger, J.H., Boyer, E., Van Dongen, C.G., and Chu, K.C.: Testing of environmental aromatic amines or derivatives for long-term toxicity or carcinogenicity. J. Toxicol. Environ. Health (In press).

113. Williams, G.M.: The detection of chemical carcinogens by unscheduled DNA synthesis in rat liver primary cell cultures. Cancer Res. 37: 1845-1851, 1977.

114. Williams, G.M.: The detection of chemical mutagens/carcinogens by DNA repair and mutagenesis in liver cultures. In DeSerres, F.J. and Hollaender, A. (Eds.): Chemical Mutagens (In press).

115. Williams, G.M.: Further improvements in the hepatocyte DNA repair test for carcinogens: Detection of carcinogenic biphenyl derivatives. Cancer Lett. (In press).

116. Williams, G.M., Bermudez, E., San, R.H.C., Goldblatt, P.J., and Laspia, M.F.: Rat hepatocyte primary cultures. IV. Maintenance in defined medium and the role of production of plasminogen activator and other proteases. In Vitro (In press).

117. Williams, G.M., Bermudez, E., and Scaramuzzino, D.: Rat hepatocyte primary cell cultures. III. Improved dissociation and attachment techniques and the enhancement of survival by culture medium. In Vitro 13: 809-817, 1977.

118. Wipke, W.T., Krishnan, S., and Ouchi, G.I.: Hash functions for rapid storage and retrieval of chemical structures. J. Chem. Info. Comput. Sci. 18: 32-37, 1978.

119. Wislocki, P.G., Miller, E.C., Miller, J.A., McCoy, E.C., and Rosenkranz, H.S.: Carcinogenic and mutagenic activities of safrole, 1'-hydroxysafrole, and some known or possible metabolites. Cancer Res. 37: 1883-1891, 1977.

120. Zardi, L., Saint Vincent, L., Barbin, A., Montesano, R., and Margison, G.P.: Effect of split doses of N-methyl-N-nitrosourea on DNA repair synthesis in cultured mammalian cells. Cancer Lett. 3: 183-188, 1977.

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VI. APPENDIX

Chemicals Being Tested for Carcinogenicity by the Carcinogenesis Testing Program, DCCP, NCI

June 1, 1978

Chemicals tested in this program are selected on the basis of human exposure, production levels and chemical structure. They should not be considered as suspected carcinogens by virtue of being in the Testing Program. Compounds are listed by their common or generic name; if this is not available, then chemical name is used. For additional information address requests to: Technical Information Resources Branch, CGT, DCCP, National Cancer Institute, Landow Building, Room 3A04, National Institutes of Health, Bethesda, Maryland 20014.

CHEMICALS ARE ARRANGED ACCORDING TO THEIR TESTING STATUS WHICH ARE:

TABLE I	DATA AVAILABLE
	IA TECHNICAL REPORTS PUBLISHED
	IB NO REPORT - DATA INSUFFICIENT
TABLE II	BIOASSAY COMPLETE - REPORTS HAVE BEEN DRAFTED
	IIA REPORTS BEING PRINTED
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TABLE IV	HISTOPATHOLOGY INCOMPLETE
TABLE V	BIOASSAY HAS BEGUN BUT NO CHRONIC TESTING DATA ARE AVAILABLE
TABLE VI	CHEMICALS THAT HAVE TENTATIVELY BEEN SELECTED FOR TESTING

ABBREVIATIONS USED IN THIS REPORT ARE AS FOLLOWS:

ROUTES OF ADMINISTRATION:

- FEED - ORALLY WITH FOOD
- GAV - ORALLY BY GAVAGE
- INHAL - INHALATION
- WATER ORALLY WITH WATER
- IJ - INJECTION
- IP - INTRAPERITONEAL
- SP - SKIN PAINTING

ANIMALS SPECIES:

- H - HAMSTER
- M - MOUSE
- R - RAT

SIMULTANEOUS CHEMICAL LISTINGS

CHEMICALS THAT APPEAR MORE THAN ONCE REPRESENT SEVERAL EXPERIMENTS.

TABLE I. DATA AVAILABLE

IA TECHNICAL REPORTS PUBLISHED

NCI #	CHEMICAL NAME	CAS #	ROUTE	SPECIES	
C03974	ACETIC ACID, (ETHYLENEDINITRILO)TETRA, TRISODIUM SALT	150-38-9	FEED	M	R
C02766	ACETIC ACID, NITRILOTRI-	139-13-9	FEED	M	R
C01445	ACETIC ACID, NITRILOTRI-, TRISODIUM SALT, MONOHYDRATE	18662-53-8	FEED	M	R
C01445	ACETIC ACID, NITRILOTRI-, TRISODIUM SALT, MONOHYDRATE	18662-53-8	FEED	M	R
C04137	ACRIDINE, 3,6-DIAMINO-,MONOHYDROCHLORIDE	952-23-8	FEED	M	R
C00044	ALDRIN	309-00-2	FEED	M	R
C01730	ANTHRANILIC ACID	118-92-3	FEED	M	R
C01923	ANTHRAQUINONE, 2-METHYL-1-NITRO-	129-15-7	FEED	M	R
C00077	CAPTAN	133-06-2	FEED	M	R
C00055	CHLORAMBEN	133-90-4	FEED	M	R
C00099	CHLORDANE	57-74-9	FEED	M	R
C00191	CHLORDECONE	143-50-0	FEED	M	R
C02686	CHLOROFORM	67-66-3	GAV	M	R
C01718	DAPSONE	80-08-0	FEED	M	R
C03269	DIARYLANILIDE YELLOW	6358-85-6	FEED	M	R
C00113	DICHLORVOS	62-73-7	FEED	M	R
C00124	DIELDRIN	60-57-1	FEED	M	R
C00124	DIELDRIN	60-57-1	FEED		R
C00599	DIELDRIN, PHOTO-	13366-73-9	FEED	M	R
C00135	DIMETHOATE	60-51-5	FEED	M	R
C01605	EMETINE	483-18-1	IP/IJ	M	R
C04626	ETHANE, 1,1,1-TRICHLORO-	71-55-6	GAV	M	R
C04580	ETHYLENE, TETRACHLORO-	127-18-4	GAV	M	R
C00180	HEPTACHLOR	76-44-8	FEED	M	R
C01547	IPD	3458-22-8	IP/IJ	M	R
C01638	ISOPHOSPHAMIDE	3778-73-2	IP/IJ	M	R
C01478	LASIOCARPINE	303-34-4	FEED	R	
C00204	LINDANE	58-89-9	FEED	M	R
C00215	MALATHION	121-75-5	FEED	M	R
C00497	METHOXYCHLOR	72-43-5	FEED	M	R
C00420	NITROFEN	1836-75-5	FEED	M	R
C01741	PHENFORMIN	114-86-3	FEED	M	R
C00237	PICLORAM	1918-02-1	FEED	M	R
C00500	PROPANE, 1,2-DIBROMO-3-CHLORO-	96-12-8	GAV	M	R
C03554	TETRACHLOROETHANE	79-34-5	GAV	M	R
C04546	TRICHLOROETHYLENE	79-01-6	GAV	M	R
C00168	TETRACHLORVINPHOS	961-11-5	FEED	M	R
C01763	UREA, 1-BUTYL-3-(P-TOLYLSULFONYL)-	64-77-7	FEED	M	R
C01752	UREA, 1-((P-CHLOROPHENYL)SULFONYL)-3-PROPYL-	94-20-2	FEED	M	R

TABLE I. DATA AVAILABLE

IA TECHNICAL REPORTS PUBLISHED

CHEMICALS MOVED FROM TABLE IIA SINCE LAST ISSUE.

NCI #	CHEMICAL NAME	CAS #	ROUTE	SPECIES	
C01707	ANILINE, 4,4'-THIODI-	139-65-1	FEED	M	R
C01569	5-AZACYTIDINE	320-67-2	IP/IJ	M	P
C02664	BIPHENYL, CHLORO-	27323-18-8	FEED	O	
C01989	2,4-DIAMINOANISOLE SULFATE	39156-41-7	FEED	M	P
C02653	HEXACHLOROPHENE	70-30-4	FEED	O	
C01785	PYRAZINECARBOXAMIDE	98-96-4	FEED	M	P
C00442	TRIFLURALIN	1582-09-8	FEED	M	P
C03270	TRIS(2,3-DIBROMOPROPYL)PHOSPHATE	126-72-7	FEED	M	P

TABLE I. DATA AVAILABLE

IB NO REPORT - DATA INSUFFICIENT

NCI #	CHEMICAL NAME	CAS #	ROUTE	SPECIES	
C04682	ACTINOMYCIN D	50-76-0	IP/IJ	M	P
C04728	ARABINOSYLCYTOSINE	147-94-4	IP/IJ	M	P
C03474	AZATHIOPRINE	446-86-6	IP/IJ	M	P
C01592	1,4-BUTANEDIOL, DIMETHANESULFONATE	55-98-1	IP/IJ	M	P
C04808	CARBANILIDE, 4,4' DIACETYL-BIS(AMIDINOHYDRAZONE),DIMETHANE	15427-93-7	IP/IJ	M	F
C03485	CHLORAMBUCIL	305-03-3	IP/IJ	M	F
C04900	CYCLOPHOSPHAMIDE	50-18-0	IP/IJ	M	P
C04693	DAUNOMYCIN	1407-15-4	IP/IJ	M	F
C04933	O,P'-DDD	53-19-0	IP/IJ	M	F
C03667	DIBENZO-P-DIOXIN, 2,7-DICHLORO-	33857-26-0	FEED	M	R
C03678	DIBENZO-P-DIOXIN, 1,2,3,4,6,7,8,9-OCTACHLORO-	3268-87-9	FEED	M	P
C03656	DIBENZO-P-DIOXIN	262-12-4	FEED	M	P
C04795	DIBROMODULCITOL	10318-26-0	IP/IJ	M	F
C04762	DIBROMOMANNITOL	488-41-5	IP/IJ	M	F
C03689	P-DIOXANE	123-91-1	WATER	M	P
C04819	GUANAZOLE	503-88-8	IP/IJ	M	P
C03054	HYDANTOIN, DICHLORODIMETHYL-	118-52-5	FEED	M	R
C04739	HYDRAZINE, 1-ACETYL-2-PICOLINOYL-	17433-31-7	IP/IJ	M	R
C04831	HYDROXYLUREA	127-07-1	IP/IJ	M	R
C04717	IMIDAZOLE-4-CARBOXAMIDE-5-(3,3-DIMETHYL-1-TRIAZENO)-	4342-03-4	IP/IJ	M	R
C01616	IMIDAZOLE MUSTARD	5034-77-5	IP/IJ	M	P
C04853	MELPHALAN	148-82-3	IP/IJ	M	P
C04886	6-MERCAPTOPURINE	50-44-2	IP/IJ	M	R
C04944	MERPHALAN		IP/IJ	M	F
C03190	METHANSULFON-M-ANISIDE, 4'-(9-ACRIDINYLAMINO)-, MONOHYDRO		IP/IJ	M	P
C04671	METHOTREXATE	59-05-2	IP/IJ	M	F
C04875	METHOTREXATE, DICHLORO-	528-74-5	IP/IJ	M	P
C04784	METHYLTHIOINOSINE	342-69-8	IP/IJ	M	F
C04706	MITOMYCIN C	50-07-7	IP/IJ	M	F
C04922	PHOSPHORODIAMIDIC ACID, N,N-BIS(2-CHLOROETHYL)-N'-3-HYDROXY		IP/IJ	M	P
C04897	PREDNISONE	53-03-2	IP/IJ	M	P
C01810	PROCARBAZINE HYDROCHLORIDE	366-70-1	IP/IJ	M	F
C03167	STREPTOZOTICIN	18883-66-4	IP/IJ	M	F
C04820	URACIL MUSTARD	66-75-1	IP/IJ	M	F
C04773	UREA, 1,3-BIS(CHLOROETHYL)-1-NITROSO	154-93-8	IP/IJ	M	F
C04740	UREA, 1-(2-CHLOROETHYL)-3-CYCLOHEXYL-1-NITROSO-	13010-47-4	IP/IJ	M	F
C04955	UREA, N-(2-CHLOROETHYL)-N'-(4-METHYLCYCLOHEXYL)-N-NITROSO-		IP/IJ	M	R
C04842	VINBLASTINE	865-21-4	IP/IJ	M	P
C04864	VINCRISTINE	57-22-7	IP/IJ	M	P

TABLE II. BIOASSAY COMPLETE · REPORTS HAVE BEEN DRAFTED

IIA REPORTS BEING PRINTED

NCI #	CHEMICAL NAME	CAS #	ROUTE	SPECIES	
C01887	ACETANILIDE, 3-AMINO-4-ETHOXY-		FEED	M	F
C01536	ACRONYCINE	7008-42-6	IP/IJ	M	R
C02993	P-ANISIDINE, 2-METHYL	102 50-1	GAV	M	F
C01901	ANTHRAQUINONE, 1-AMINO-2-METHYL-	82-28-0	FEED	M	R
C02697	ASPIRIN, PHENACETIN AND CAFFEINE	8003-03-0	FEED	M	R.
C00419	BENZENE, PENTACHLORONITRO-	82-68-8	FEED	M	P
C00533	CHLOROPICRIN	76-06-2	GAV	M	F
C01558	CHOLESTEROL, (P-(BIS(2-CHLOROETHYL)AMINO)PHENYL) ACETATE	3546-10-9	GAV	M	F
C01693	DARAPRIM	58-14-0	FEED	M	R
C01581	BETA-DEOXYTHIOGUANOSINE	789-61-7	IP/IJ	M	F
C01661	DIBENZYLINE HYDROCHLORIDE	63-92-3	IP/IJ	M	P
C03689	P-DIOXANE	123-91-1	WATER	M	P
C54557	DIRECT BLACK 38	1937-37-7	FEED	M	P
C54579	DIRECT BLUE 6		FEED	M	F
C54568	DIRECT BROWN 95	10300-74-0	FEED	M	F
C00566	ENDOSULFAN	115-29-7	FEED	M	F
C04535	ETHANE, 1,1-DICHLORO-	75-34-3	GAV	M	R
C00511	ETHANE, 1,2-DICHLORO-	107-06-2	GAV	M	R
C04604	ETHANE, HEXACHLORO-	67-72-1	GAV	M	F
C04579	ETHANE, 1,1,2-TRICHLORO-	79-00-5	GAV	M	P
C01694	ETHIONAMIDE	536-33-4	FEED	M	R
C03258	HYDROXYLAMINE, N-NITROSO-N-PHENYL-, AMMONIUM SALT	135-20-6	FEED	M	R
C50000	MENTHOL,DL	89-78-1	FEED	M	R

TABLE II. BIOASSAY COMPLETE -- REPORTS HAVE BEEN DRAFTED

IIA REPORTS BEING PRINTED

NCI #	CHEMICAL NAME	CAS #	ROUTE	SPECIES	
C01956	NAPHTHALENE, 1-NITRO-	86-57-7	FEED	M	F
C03305	M-PHENYLENEDIAMINE, 4 CHLORO	5131-60-2			
C03292	O-PHENYLENEDIAMINE, 4-CHLORO-	95-83-0	FEED	M	P
C02233	P-PHENYLENEDIAMINE, N-PHENYL-, HYDROCHLORIDE	2198-59-6	FEED	M	P
C01627	2,6-PIPERAZINEDIONE-4,4'-PROPYLENE DIOXOPIPERAZINE		IP/IJ	M	P
C03076	PROPIONIC ACID, 3-NITRO-	504-88-1	GAV	M	F
C03827	SUCCINIC ACID, MONO(2,2-DIMETHYLHYDRAZIDE)-	1596-84-5	FEED	M	F
C03065	THIAZOLE, 2-AMINO-5-NITRO-	121-66-4	FEED	M	F
C04557	THIOPHENE, 2,5-DIHYDRO-, 1,1-DIOXIDE-	77-79-2	GAV	M	F
C01865	TOLUENE, 2,4-DINITRO-	121-14-2	FEED	M	F
C03781	TRIMETHYL PHOSPHATE	512-56-1	FEED	M	P
C01729	TRYPTOPHAN, L-	73-22-3	FEED	M	F
C03247	UREA, 1-((P-ACETYLPHENYL)SULFONYL)-3-CYCLOHEXYL-	968-81-0	FEED	M	R
C04524	UREA, N,N'-DICYCLOHEXYLTHIO-	1212-29-9	FEED	M	P
C03327	UREA, 1-(HEXAHYDRO-1H-AZEPIN-1-YL)-3-(P-TOLYLSULFONYL)-	1156-19-0	FEED	M	R

CHEMICALS MOVED FROM TABLE IIB SINCE LAST ISSUE

NCI #	CHEMICAL NAME	CAS #	ROUTE	SPECIES	
C04615	ALLYL CHLORIDE	107-05-1	GAV	M	P
C01945	ANTHRANILIC ACID, 4-NITRO-	619-17-0	FEED	M	P
C03521	1H-BENZOTRIAZOLE	95-14-7	FEED	M	R
C00395	DIOXATHION	78-34-2	FEED	M	R
C01570	ESTRADIOL MUSTARD		GAV	M	P
C00522	ETHANE, 1,2-DIBROMO-	106-93-4	GAV	M	F
C04568	METHANE, TRIIODO-	75-47-8	GAV	M	P
C00431	NICLOSAMIDE	1420-04-8	FEED	M	R
C03316	P-PHENYLENEDIAMINE, 2-CHLORO-	615-66-7	FEED	M	P
C01672	PYRIDIUM	136-40-3	FEED	M	P
C01649	THIO TEPA	52-24-4	IP/IJ	M	P

TABLE II. BIOASSAY COMPLETE -- REPORTS HAVE BEEN DRAFTED

IIB REPORTS IN REVIEW

NCI #	CHEMICAL NAME	CAS #	ROUTE	SPECIES	
C01967	ACENAPHTHENE, 5-NITRO-	602-87-9	FEED	M	R
C02103	ACETAMIDE	60-35-5	FEED	M	R
C01978	P-ACETOPHENETIDE, 3'-NITRO-		FEED	M	R
C03349	ACETOHYDROXAMIC ACID, N-FLOUREN-2-YL-	53-95-2	FEED	R	
C08640	ALDICARB	116-06-3	FEED	M	R
C01821	ANILINE, N,N-DIMETHYL-P-NITROSO-	138-89-6	FEED	M	R
C03747	O-ANISIDINE HYDROCHLORIDE	134-29-2	FEED	M	R
C03758	P-ANISIDINE HYDROCHLORIDE	20265-97-8	FEED	M	R
C01934	O-ANISIDINE, 5-NITRO-	99-59-2	FEED	M	R
C03032	ANISOLE, 2,3,5,6-TETRACHLORO-4-NITRO-	2438-88-2	FEED	M	R
C01876	ANTHRAQUINONE, 2-AMINO	117-79-3	FEED	M	R
C02120	L-ARGININE GLUTAMATE	4320-30-3	FEED	M	R
C00066	AZINPHOS-METHYL	86-50-0	FEED	M	R
C01912	BENZIMIDAZOLE, 6-NITRO-	94-52-0	FEED	M	R
C02131	N-BUTYLUREA	592-31-4	FEED	M	R
C03043	CARBAZOLE, 3-AMINO-9-ETHYL-	132-32-1	FEED	M	R
C01898	CARBAZOLE, 3-AMINO-9-ETHYL-, HYDROCHLORIDE		FEED	M	R
C04591	CARBON DISULFIDE	75-15-0	GAV	M	R
C00408	CHLOROBENZILATE	510-15-6	FEED	M	R
C03838	3-(CHLOROMETHYL)-PYRIDINE HYDROCHLORIDE		GAV	M	R
C02982	P-CRESIDINE	120-71-8	FEED	M	R
C00102	DACONIL	1897-45-6	FEED	M	R
C00475	P,P'-DDD	72-54-8	FEED	M	R
C00555	P,P'-DDE	72-55-9	FEED	M	R
C00464	P,P'-DDT	50-29-3	FEED	M	R
C00486	DICOFOL	115-32-2	FEED	M	R
C03009	2,5-DITHIOBIUREA	142-46-1	FEED	M	R
C00157	ENDRIN	72-20-8	FEED	M	R

TABLE II. BIOASSAY COMPLETE -- REPORTS HAVE BEEN DRAFTED

IIB REPORTS IN REVIEW

NCI #	CHEMICAL NAME	CAS #	ROUTE	SPECIES	
C01854	HYDRAZINE, 1,2-DIPHENYL-	122-66-7	FEED	M	R
C04637	METHANE, TRICHLOROFLUORO-	75-69-4	GAV	M	R
C03849	METHIODAL SODIUM	126-31-8	IP/IJ	M	R
C03010	METHYL ORANGE B	547-58-0	FEED	M	R
C00544	HEXACARBATE	315-18-4	FEED	M	R
C03021	1,5-NAPHTHALENEDIAMINE	2243-62-1	FEED	M	R
C02084	NITROUS ACID, SODIUM SALT	7632-00-0	FEED	M	R
C03952	NORPHENAZONE	89-25-8	FEED	M	R
C04126	2-OXETANONE, 3,3-DIMETHYL-	1955-45-9	GAV	M	R
C00276	PARATHION	56-38-2	FEED	M	R
C03963	PHENOL,4-AMINO-2-NITRO-	119-34-6	FEED	M	R
C03930	P-PHENYLENEDIAMINE, DIHYDROCHLORIDE	624-18-0	FEED	M	R
C00588	PHOSPHAMIDON	13171-21-6	FEED	M	R
C01810	PROCARBAZINE HYDROCHLORIDE	366-70-1	IP/IJ	M	R
C00453	SULFALLATE	95-06-7	FEED	M	R
C50022	SULFISONAZOLE	127-69-5	GAV	M	R
C04240	TITANIUM DIOXIDE	13463-67-7	FEED	M	R
C01832	TOLUENE-2,5-DIAMINE, SULFATE	6369-59-1	FEED	M	R
C02040	P-TOLUIDINE, 3-CHLORO-	95-74-9	FEED	M	R
C01843	O-TOLUIDINE, 5-NITRO-	99-55-8	FEED	M	R
C02153	P-TOLYLUREA	622-51-5	FEED	M	R
C00259	TOXAPHENE	8001-35-2	FEED	M	R
C00260	TRIPHENYLTIN HYDROXIDE	76-87-9	FEED	M	R
C02119	UREA	57-13-6	FEED	M	R
C03816	UREA, 1,3-DIETHYL-2-THIO-	105-55-5	FEED	M	R
C02073	UREA, (P-ETHOXYPHENYL)-	150-69-6	FEED	M	R
C02017	UREA, 1-PHENYL-2-THIO-	103-85-5	FEED	M	R

CHEMICALS MOVED FROM TABLE III SINCE LAST ISSUE

NCI #	CHEMICAL NAME	CAS #	ROUTE	SPECIES	
C02175	ISOCYANIC ACID, 3,3'-DIMETHOXY-4,4'-BIPHENYLENE ESTER	91-93-0	FEED	M	R
C03792	NITHIAZIDE	139-94-6	FEED	M	R
C02211	STYRENE, BETA-NITRO-	102-96-5	GAV	M	R
C02186	UREA, 1,1,3-TRIMETHYL-2-THIO-	2489-77-2	FEED	M	R

TABLE IIIA. BIOASSAY HAS BEEN COMPLETED -- REPORTS ARE BEING DRAFTED

NCI #	CHEMICAL NAME	CAS #	ROUTE	SPECIES
C03770	ACETANILIDE, 4' (CHLOROACETYL)-	140-49-8	FEED	M R
C02095	ADIPAMIDE	628-94-4	FEED	M R
C03736	ANILINE CHLORIDE	142-04-1	FEED	M F
C02039	ANILINE, P-CHLORO	106-47-8	FEED	M F
C02255	ANILINE, 2,4-DIMETHOXY-, HYDROCHLORIDE		FEED	M P
C01990	ANILINE, 4,4'-METHYLENEBIS (N,N-DIMETHYL)-	101-61-1	FEED	M P
C08684	ANILIZIDE	101-05-3	FEED	M F
C02006	BENZOPHENONE, 4,4'-BIS(DIMETHYLAMINO)-	90-94-8	FEED	M P
C03850	P-BENZOQUINONE, DIOXIME	105-11-3	FEED	M F
C03861	5BETA-CHOLAN-24-OIC ACID, 3ALPHA-HYDROXY-	434-13-9	GAV	M F
C03565	C.I. VAT YELLOW 4	128-66-5	FEED	M P
C08662	COUMAPHOS	56-72-4	FEED	M P
C03667	DIBENZO-P-DIOXIN, 2,7-DICHLORO-	33857-26-0	FEED	M F
C03656	DIBENZO-P-DIOXIN	262-12-4	FEED	M P
C02028	DIBUTYLTIN DIACETATE	1067-33-0	FEED	M R
C50055	DIMETHYL TEREPHTHALATE	120-61-6	FEED	M F
C02244	DIPHENYLAMINE, 4-NITROSO-	156-10-5	FEED	M F
C50044	ETHER, BIS(2-CHLORO-1-METHYLETHYL)	108-60-1	GAV	M
C02857	ETHYL TELLURAC	30145-38-1	FEED	M F
C08651	FENTHION	55-38-9	FEED	M F
C02142	HEXANAMIDE	628-02-4	FEED	M R
C03281	N-1-NAPHTHYLETHYLENEDIAMINE DIHYDROCHLORIDE	1465-25-4	FEED	M P
C00420	NITROFEN	1836-75-5	FEED	M P
C03941	O-PHENYLENEDIAMINE, 4-NITRO-	99-56-9	FEED	M P
C02222	P-PHENYLENEDIAMINE, 2-NITRO-	5307-14-2	FEED	M P
C02813	PIPERONYL BUTOXIDE	51-03-6	FEED	M F
C02824	PIPERONYL SULFOXIDE	120-62-7	FEED	M P
C03907	PYRIDINE, 2-(CHLOROMETHYL)-, HYDROCHLORIDE		GAV	M F
C02200	STYRENE	100-42-5	GAV	M F
C02051	O-TOLUIDINE, 5-CHLORO	95-79-4	FEED	M P
C03805	UREA, (2-BROMO-2-ETHYLBUTYRYL)-	77-65-6	FEED	M P

CHEMICALS MOVED FROM TABLE IV SINCE LAST ISSUE

TABLE IIIB. BIOASSAY HAS BEEN COMPLETED

NCI #	CHEMICAL NAME	CAS #	ROUTE	SPECIES	
POSITIVE CONTROL					
C00384	ACETAMIDE, N-FLUOREN-2-YL-	53-96-3	FEED	M	R
C01456	AFLATOXIN B1	1162-65-8	FEED	R	
C01434	1-BUTANOL, 4-(BUTYLNITROSAMINO)-	3817-11-6	FEED	R	
C03452	CARBON TETRACHLORIDE	56-23-5	GAV	M	R
C01467	CYCASIN	14901-08-7	FEED	R	
C02700	DIPENTYLAMINE, N-NITROSO-	13256-06-9	FEED	R	
C01423	GUANIDINE, 1-METHYL-3-NITRO-1-NITROSO-	70-25-7	FEED	R	
C01489	LEAD ACETATE	301-04-2	FEED	R	
C00362	SAFROLE	94-59-7	FEED	R	M
C00373	S-TRIAZOLE, 3-AMINO-	61-82-5	FEED	M	R
C01490	URACIL, 2-THIO-	141-90-2	FEED	R	

TABLE IV. HISTOPATHOLOGY INCOMPLETE

NCI #	CHEMICAL NAME	CAS #	ROUTE	SPECIES	
C01514	ADRIAMYCIN	23214-92-8	IP/IJ	M	P
C02960	AMMONIUM, (2-CHLOROETHYL)TRIMETHYL-, CHLORIDE	999-81-5	FEED	M	R
C02299	ANILINE, 2,4,5-TRIMETHYL-	137-17-7	FEED	M	P
C02926	AZOBENZENE	103-33-3	FEED	M	F
C50011	BENZOIN	119-53-9	FEED	M	P
C02937	CALCIUM CARBIMIDE	156-62-7	FEED	M	P
C03510	CINNAMYL ANTHRANILATE	87-29-6	FEED	M	F
C03598	P-CRESOL, 2,6-DI-TERT-BUTYL-	128-37-0	FEED	M	F
C08673	DIAZINON	333-41-5	FEED	M	P
C03703	DIBENZO-P-DIOXIN, 1,2,3,6,7,8-HEXACHLORO-		SP	M	
C03703	DIBENZO-P-DIOXIN, 1,2,3,6,7,8-HEXACHLORO-		GAV	M	R
C03714	DIBENZO-P-DIOXIN, 2,3,7,8-TETRACHLORO	1746-01-6	SP	M	
C03714	DIBENZO-P-DIOXIN, 2,3,7,8-TETRACHLORO-	1746-01-6	GAV	M	P
C02880	DIPHENYLAMINE, N-NITROSO-	86-30-6	FEED	M	P
C02868	ETHANE, 1,1-DICHLORO-2,2-BIS(P-ETHYLPHENYL)-	72-56-0	FEED	M	R
C02959	ETHYL TUADS	14239-68-0	FEED	M	P
C08695	FLUOMETURON	2164-17-2	FEED	M	
C50259	HEXAMETHYLMELAMINE	645-05-6	IP/IJ	M	P
C02891	LEDATE	19010-66-3	FEED	M	P
C00215	MALATHION	121-75-5	FEED	M	P
C08628	MALATHION-O-ANALOG	1634-78-2	FEED	M	F
C53838	ORANGE G	1936-15-8	FEED	P	
C50124	PHENOL	108-95-2	WATER	M	
C50124	PHENOL	108-95-2	WATER	P	
C02904	PHENOL, 2,4,6-TRICHLORO-	88-06-2	FEED	M	F
C02971	PHOSPHOROTHIOIC ACID, O,O-DIMETHYL O-(P-NITROPHENYL) ESTER	298-00-0	FEED	M	P
C03612	PHTHALAMIDE	88-96-0	FEED	M	R
C03601	PHTHALIC ANHYDRIDE	85-44-9	FEED	M	P
C00033	1,2-PROPANEDIOL	57-55-6	SP	M	R
C50157	RESERPINE	50-55-5	FEED	M	P
C50033	SELENIUM SULFIDE	7488-56-4	GAV	M	R
C54546	SELSUN		SP	M	
C02835	SODIUM DIMETHYLDITHIOCARBAMATE	128-04-1	FEED	M	F
C53907	SUN YELLOW FCF	2783-94-0	FEED	P	
C02302	TOLUENE-2,4-DIAMINE	95-80-7	FEED	M	P
C02368	O-TOLUIDINE, 4-CHLORO-, HYDROCHLORIDE	3165-93-3	FEED	M	P
C02335	O-TOLUIDINE HYDROCHLORIDE	636-21-5	FEED	M	P

TABLE V. BIOASSAY TESTING HAS BEGUN BUT NO DATA ARE AVAILABLE

NCI #	CHEMICAL NAME	CAS #	ROUTE	SPECIES	
C05732	AEROZINE 50	57 14 7	INHAL	M	
C50475	AGAR AGAR	9002-18-0	FEED	M	R
C08899	AGARITINE	2757-90-6	WATER	M	F
C50464	ALLYL ISOTHIOCYANATE	57-06-7	GAV	M	R
C50613	AMINO UNDECANOIC ACID	27323-47-3	FEED	M	F
C06360	BENZYL CHLORIDE	100-44-7	IP/IJ	M	
C50282	2-BIPHENYLAMINE	90-41-5	FEED	M	F
C50635	BISPHENOL A	80-05-7	FEED	M	R
C50602	1,3-BUTADIENE	106-99-0	INHAL	M	F
C54375	BUTYL BENZYL PHTHALATE	85-68-7	FEED	M	F
C50646	CAPROLACTAM	105-60-2	FEED	M	R
C53849	CARMOISINE	3567-69-9	FEED	M	
C08639	GAMMA-CHLORDANE	12789-03-6	FEED	M	F
C06871	CHLORDANE, ALPHA ISOMER	5103-71-9	FEED	M	R
C53781	C.I. DISPERSE YELLOW 3	2832-40-8	FEED	M	F
C55005	CYCLOHEXANONE	108-94-1	WATER	M	F
C53792	D AND C RED NO. 9	5160-02-1	FEED	M	F
C50657	DIALLYPHTHALATE	131-17-9	GAV	M	R
C50260	1,4-DIAMINO-2,6-DICHLOROBENZENE		FEED	M	R
C51581	CIS-1,2-DICHLOROETHYLENE	156 59 2	GAV	M	R
C54591	TRANS-1,2-DICHLOROETHYLENE		GAV	M	R
C54262	1,1-DICHLOROETHYLENE	75-35-4	GAV	M	F
C54386	DI(2-ETHYLHEXYL)ADIPATE	103-23-1	FEED	M	R
C52733	DI(2-ETHYLHEXYL)PHTHALATE	117-81-7	FEED	M	R
C50668	4,4'-DIPHENYLMETHANE DIISOCYANATE	101-68-8	GAV	M	R
C00522	ETHANE, 1,2-DIBROMO-	106-93-4	INHAL	M	F
C04626	ETHANE, 1,1,1-TRICHLORO-	71-55-6	GAV	M	R
C50384	ETHYL ACRYLATE	140-88-5	GAV	M	F
C50135	ETHYLENE CHLOROHYDRIN	107-07-3	SP	M	
C50453	EUGENOL	97-53-0	FEED	M	R
C50395	GUAR GUM	9000-30-0	FEED	M	R
C50748	GUM ARABIC	9000-01-5	FEED	M	F
C54364	GUM TARA		FEED	R	
C06893	HEPTACHLOREPOXIDE	1024-57-3	FEED	M	R
C50419	LOCUST BEAN GUM	9000-40-2	FEED	M	R
C50362	MANNITOL	69-65-8	FEED	M	R
C50715	MELAMINE	108-78-1	FEED	M	F
C09018	METHAPYRILENE	91-80-5	FEED	M	R
C54604	4,4'-METHYLENEDIANILINE 2HCL	101-77-9	FEED	M	R
C50102	METHYLENE CHLORIDE	75-09-2	GAV	M	R
C50680	METHYL METHACRYLATE	80-62-6	INHAL	M	R
C06428	MIREX	2385-85-5	FEED	M	R
C08902	NITROFURANTOIN	67-20-9	FEED	M	R

TABLE V. BIOASSAY TESTING HAS BEGUN BUT NO DATA ARE AVAILABLE

NCI #	CHEMICAL NAME	CAS #	ROUTE	SPECIES	
C53838	ORANGE G	1936-15-8	FEED	M	
C50146	4,4'-OXYDIANILINE	101-80-4	FEED	M	F
C53894	PENTACHLOROETHANE	76-01-7	GAV	[illegible]	
C50124	PHENOL	108-95-2	INHAL	H	
C55414	PHENYLBUTAZONE		WATER	M	F
C50077	PROPYLENE	115-07-1	INHAL	M	F
C50099	PROPYLENE OXIDE	75-56-9	INHAL	M	F
C50588	PROPYL GALLATE	121-79-9	FEED	M	F
C04035	RANDOX	93-71-0	FEED	M	F
C02722	STANNOUS CHLORIDE	7772-99-8	FEED	M	F
C54977	STYRENE OXIDE	96-09-3	GAV	M	F
C53929	SUDAN I	842-07-9	FEED	M	R
C53907	SUN YELLOW FCF	2783-94-0	FEED	M	
C03985	TELONE	542-75-6	GAV	M	P
C52459	1,1,1,2-TETRACHLOROETHANE		GAV	F	
C50317	TOLUENE-2,6-DIAMINE DIHYDROCHLORIDE		FEED	M	F
C50533	TOLUENE 2,4-DIISOCYANATE	584-84-9	GAV	M	F
C04546	TRICHLOROETHYLENE	79-01-6	GAV	M	
C50226	ZEARALENONE	7645-23-0	FEED	M	R
C50442	ZIRAM	137-30-4	FEED	M	F

TABLE VI. CHEMICALS THAT HAVE BEEN TENTATIVELY SELECTED FOR TESTING:

NCI #	CHEMICAL NAME	CAS #	ROUTE	SPECIES
C54911	ACID ORANGE #3		FEED	
C50215	ACRYLONITRILE	107-13-1		
C54717	ALLYL ISOVALERATE	2835-39-4	GAV	
C55458	1-AMINO-2,4-DIBROMOANTHRAQUINONE			
C55710	AMPHETAMINE			
C50179	AMYL NITRITE	110-46-3	GAV	
C55152	ANTIMONY OXIDE	1309-64-4		
C54908	ASCORBIC ACID	50-81-7	FEED	
C55276	BENZENE	71-43-2	GAV	
C06508	BENZYL ACETATE	140-11-4	GAV	
C06111	BENZYL ALCOHOL			
C55516	2,2-BIS(BROMOETHYL)-1,3-PROPANEDIOL			
C04159	N',N'-BIS(2-HYDROXYETHYL)-N-METHYL-2-NITRO-P-PHENYLENEDI			
C54615	BLUE 15B	574-93-6	FEED	
C55492	BROMOBENZENE			
C55243	BROMODICHLOROMETHANE	75-27-4	GAV	
C55130	BROMOFORM	75-25-2	GAV	
C50522	1,3-BUTADIENE, 2-CHLORO-	126-99-8		
C55447	2-BUTANONE PEROXIDE			
C55367	T-BUTYL ALCOHOL	75-65-0	WATER	
C55163	CASTOR OIL	8001-79-4	FEED	
C55072	CHLORENDIC ACID	115-28-6		
C55754	CHLORINATED TRISODIUM PHOSPHATE			
C55107	CHLOROACETOPHENONE (CN)	532-27-4		
C55709	CHLOROAMPHENICOL			
C55118	CHLOROBENZALMALONONITRILE AND HEXAMETHYLDISILIZANE (CS2)			
C54886	CHLOROBENZENE	108-90-7		
C55254	CHLORODIBROMOMETHANE	124-48-1	GAV	
C54820	3-CHLORO-2-METHYLPROPENE	563-47-3	GAV	
C53543	CHLOROWAX 40	51990-12-6		
C53587	CHLOROWAX 500C	56509-64-9		
C55265	CHLORPHENIRAMINE MALEATE	113-92-8	FEED	
	CHLORPROMAZINE			
C55312	COCONUT OIL ACID DIETHANOLAMINE		SP	
C50737	CYTEMBENA	2126-70-7	IP/IT	
C55287	DECABROMODIPHENYL OXIDE	1163-19-5		
C50168	DEODORIZED WINTERIZED COTTONSEED OIL			

TABLE VI. CHEMICALS THAT HAVE BEEN TENTATIVELY SELECTED FOR TESTING:

NCI #	CHEMICAL NAME	CAS #	ROUTE	SPECIES
C54795	DFM			
C55436	2,3-DIBROMO-1-PROPANOL			
C54944	O-DICHLOROBENZENE	95-50-1	GAV	
C54955	P-DICHLOROBENZENE	106-46-7	GAV	
C50704	DICHLORODIMETHYLSILANE	75-78-5		
C55345	2,4-DICHLOROPHENOL	120-83-2	FEED	
C55141	1,2-DICHLOROPROPANE	78-87-5	GAV	
C55174	DIETHANOLAMINE	111-42-2	WATER	
C00271	DIETHYLNITROSAMINE	55-18-5		H
C54966	DIGLYCIDYLRESORCINOL ETHER			
C55129	N,N-DIMETHYLDODECYLAMINE OXIDE	1643-20-5		
C54773	DIMETHYLHYDROGENPHOSPHITE		GAV	
C54762	DIMETHYL METHYLPHOSPHONATE		GAV	
C54740	DIMETHYLMORPHOLINOPHOSPHONATE		GAV	
C54819	DIMETHYLVINYLCHLORIDE	513-37-1	GAV	
C54900	DISPERSE BLUE #1	2475-45-8	FEED	
C54875	DODECYL ALCOHOL, ETHOXYLATED		FEED	
C55652	EPHEDRINE			
C07001	ALPHA-EPICHLOROHYDRIN	106-89-8	INHAL	H M F
C55663	EPINEPHRINE			
C55527	1,2-EPOXYBUTANE			
C55538	1,2-EPOXYHEXADECANE			
C55674	ERYTHROMYCIN			
C00522	ETHANE, 1,2-DIBROMO-	106-93-4		
C55481	ETHYL BROMIDE			
C50066	ETHYLENE	74-85-1		
C50135	ETHYLENE CHLOROHYDRIN	107-07-3	SP	R
C54853	ETHYLENE GLYCOL MONOETHYL ETHER	110-80-5	WATER	
C50088	ETHYLENE OXIDE	75-21-8	INHAL	M R
C04580	ETHYLENE, TETRACHLORO-	127-18-4	INHAL	H M F
C54706	FLUORESCEIN, DISODIUM SALT	518-47-8	WATER	
C02799	FORMALDEHYDE	50-00-0	INHAL	M R
C54728	GERANYL ACETATE	105-87-3	GAV	
C55185	GILSONITE	12002-43-6	SP	
C55425	GLUTARALDEHYDE			
C55549	GLYCIDOL			
C54897	HC BLUE #2		FEED	
C54922	HC RED #3		FEED	

TABLE VI. CHEMICALS THAT HAVE BEEN TENTATIVELY SELECTED FOR TESTING:

NCI #	CHEMICAL NAME	CAS #	ROUTE	SPECIES
C55607	HEXACHLOROCYCLOPENTADIENE			
C55298	8-HYDROXYQUINOLINE	148-24-3	FEED	
C55618	ISOPHORONE			
C55630	ISOPROTERENOL HCL			
C55323	LAURIC ACID DIETHANOLAMINE	120-40-1	SP	
C55572	D-LIMONENE			
C54648	LITHOL RUBINE	5281-04-9		
C54660	MALEIC HYDRAZIDE DIETHANOLAMINE SALT	5716-15-4	WATER	
C54842	MALONALDEHYDE	542-78-9	GAV	
C55685	ALPHA-METHYL BENZYL ALCOHOL			
C00726	20-METHYLCHOLANTHRENE	56-49-5		P
C55721	METHYL-DOPA			
C55594	METHYL CARBAMATE			
C55629	METHYL CHLORIDE			
C50102	METHYLENE CHLORIDE	75-09-2	INHAL	M P
C55550	METHAPYRILENE			
C54626	MOLYBDATE ORANGE	12213-61-5	FEED	
C02846	MONURON	150-68-5	FEED	
C02164	MORPHOLINE, 4-NITROSO-	59-89-2		
C02915	2-NAPHTHYLAMINE, N-PHENYL-	135-88-6	FEED	
C54784	NAVY FUELS JP-5 (PETROLEUM DERIVED)		SPIG	
C55049	NAVY FUELS JP-5 (SHALE OIL DERIVED)			
C00806	NITROGEN DIOXIDE	10102-44-0		H
C05050	N-NITROSODIMETHYLAMINE	62-75-9		
C55583	N-NITROSODIETHANOLAMINE			
C50180	NITROUS ACID, ETHYL ESTER	109-95-5		
C02084	NITROUS ACID, SODIUM SALT	7632-00-0		
C54580	NON-DEODORIZED WINTERIZED COTTONSEED OIL			
C55334	OLEIC ACID DIETHANOLAMINE		SP	
C55469	ORGANIDIN			
C55209	OXALIC ACID	144-62-7	FEED	
C08935	PARAFFIN OIL	8012-95-1		
C54933	PENTACHLOROPHENOL	87-86-5	FEED	
C55743	PENTAERITHRITOL TETRANITRATE			

TABLE VI. CHEMICALS THAT HAVE BEEN TENTATIVELY SELECTED FOR TESTING:

NCI #	CHEMICAL NAME	CAS #	ROUTE	SPECIES
C55641	PHENYLEPHRINE HCL	1328-53-6		
C54637	PHTHALOCYANINE GREEN	110-86-1	GAV	
C55301	PYRIDINE	8063-17-0		
C50339	RAUWOLFIA SERPENTINA			
C54739	P-ROSANILINE			
C55210	ROTENONE			
C50566	SIMETHICONE			
C55505	SODIUM ALUMINOSILICATE			
C55732	SODIUM DICHLOROISOCYANURATE			
C50191	SODIUM DODECYL SULFATE			
C50204	SODIUM(2-ETHYLHEXYL)ALCOHOL SUL			
C55221	SODIUM FLUORIDE			
C55696	SUCCINIC ANHYDRIDE			
C55561	TETRACYCLINE HYDROCHLORIDE			
C54988	TETRAETHYL LEAD			
C55061	THPC			
C55050	THPS			
C54831	TRICHLORFON			
C04546	TRICHLOROETHYLENE			
C54751	TRIS(2-ETHYLHEXYL)PHOSPHATE			
C03327	UREA, 1-(HEXAHYDRO-1H-AZEPIN-1-			
C50373	VINYLBROMIDE			
C54999	VINYLCYCLOHEXENE			
C54659	VIOLET 3			
C55470	WOLLASTONITE			
C55232	XYLENES			

CHEMICALS ADDED TO TABLE VI SINCE LAST ISSUE

CIS-DICHLORODIAMINE PLATINUM (II)
HEXYLRESORCINOL
PHENYTOIN SODIUM
PICLORAM

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